Published in final edited form as:
Toxicol Sci. 2018 June 01; 163(2): 346–352. doi:10.1093/toxsci/kfy047.

Representing the Process of Inflammation as Key Events in Adverse Outcome Pathways

Daniel L. Villeneuve[*], **Brigitte Landesmann**[†], **Paola Allavena**[‡], **Noah Ashley**[#], **Anna Bal-Price**[†], **Emanuela Corsini**[§], **Sabina Halappanavar**[¶], **Tracy Hussell**[‖], **Debra Laskin**[III], **Toby Lawrence**[¤], **David Nikolic-Paterson**[**], **Marc Pallardy**[††], **Alicia Paini**[†], **Raymond Pieters**[a], **Robert Roth**[b], and **Florianne Tschudi-Monnet**[c]

[a]IRAS-Utrecht University and University of Applied Sciences Utrecht, Yalelaan 104, 3584 CM Utrecht, The Netherlands. r.h.h.pieters@uu.nl

[b]Department of Pharmacology and Toxicology, Institute for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824. rothr@msu.edu

[c]Department of Physiology, University of Lausanne, 7, rue du Bugnon CH-1005 Lausanne, Switzerland. Florianne.tschudi-monnet@unil.ch

[*] United States Environmental Protection Agency, Office of Research and Development, Mid-Continent Ecology Division, Duluth, MN, USA 55804. Villeneuve.dan@epa.gov

[†] European Commission, Joint Research Centre, Ispra, Italy, 21027. Brigitte.LANDESMANN@ec.europa.eu; alicia.paini@ec.europa.eu; anna.price@ec.europa.eu

[‡] IRCCS Humanitas and Clinical Research Center, 20089 Rozzano, (MI), Italy. Paola.allavena@humanitasresearch.it

[#] Department of Biology, Western Kentucky University, Bowling Green, KY, USA 42101. Noah.ashley@wku.edu

[§] Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy. Emanuela.corsini@unimi.it

[¶] Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada K1A 0K9. Sabina.halappanavar@canada.ca

[‖] Collaborative Centre for Inflammation Research, University of Manchester, Manchester, United Kingdom M13 9NT. Tracy.hussell@manchester.ac.uk

[III] Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, New Jersey, USA 08854. laskin@eohsi.rutgers.edu

[¤] Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Inserm, U1104 CNRS UMR7280, Marseille, France. lawrence@ciml.univ-mrs.fr

[**] Monash University Centre for Inflammatory Diseases, Medicine Monash Health, Monash University, Clayton, Victoria, Australia. David.nikolic-paterson@monash.edu

[*]Corresponding Author: Daniel L. Villeneuve, US EPA Mid-Continent Ecology Division, 6201 Congdon Blvd, Duluth, MN, 55804, Villeneuve.dan@epa.gov, T: 218-529-5217, F: 218-529-5003.

†† Inflammation Chimiokines et Immunopathologie, INSERM, Fac. de pharmacie-Univ. Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, France. Marc.pallardy@u-psud.fr

Abstract

Inflammation is an important biological process involved in many target organ toxicities. However, there has been little consensus on how to represent inflammatory processes using the adverse outcome pathway (AOP) framework. In particular, there were concerns that inflammation was not being represented in a way that it would be recognized as a highly connected, central node within the global AOP network. The consideration of salient features common to the inflammatory process across tissues was used as a basis to propose three hub key events for use in AOP network development. Each event, “tissue resident cell activation”, “increased pro-inflammatory mediators”, and “leukocyte recruitment/activation” is viewed as a hallmark of inflammation, independent of tissue, and can be independently measured. Using these proposed hub key events it was possible to link together a series of AOPs, that previously had no shared key events. Significant challenges remain with regard to accurate prediction of inflammation-related toxicological outcomes even if a broader and more connected network of inflammation-centered AOPs is developed. Nonetheless the current proposal addresses one of the major hurdles associated with representation of inflammation in AOPs and may aid fit-for-purpose evaluations of other AOPs operating in a network context.

Keywords

networks; cell activation; damage repair; knowledge management; adverse outcome pathway

Introduction

Framing the Problem

The adverse outcome pathway (AOP) framework was developed to help organize existing knowledge concerning the linkage between stressor-induced perturbation of biological pathways and adverse outcomes considered relevant to risk assessment and regulation (Ankley et al. 2010). As such, AOPs are envisioned as an important component of a new toxicity testing paradigm that is expected to increasingly rely on mechanistic data, generally measured at low levels of biological organization using high throughput approaches, as a foundation for chemical safety assessment (Kleinstreuer et al. 2016). Likewise, AOPs are envisioned to have application in chemical category formation, design of integrated approaches to testing and assessment, and development of computational toxicity prediction models (Wittwehr et al. 2017; Edwards et al. 2016). An international AOP development program, coordinated through the Organization for Economic Cooperation and Development (OECD) and facilitated through description and dissemination of AOPs via an open access knowledgebase (aopwiki.org; aopkb.org) has emerged to support these efforts (Edwards et al. 2016; http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm).

The AOP framework, implemented via the AOP knowledgebase, employs a modular structure in which information is organized concerning measurements of biological state that

reflect progression from the initial perturbation toward the adverse outcome (key events, KEs), and scientific evidence supporting the linkage from one KE to the next in a causal and predictive sequence (key event relationships, KERs; Villeneuve et al. 2014a). This modular structure is employed so that descriptions of common elements (KEs or KERs) can be shared among multiple AOPs in order to increase the efficiency of development and to broaden the scope of their application by allowing for de facto construction of AOP networks from independently described AOPs. An AOP network is defined as a system of two or more AOPs that share one or more KEs (Knapen et al. 2017). Because it is expected that real-world exposures will often involve multiple AOPs, the ability to visualize and evaluate AOPs in a network context is important for many potential applications (Villeneuve et al. 2014a; Knapen et al. 2017). For example, one of the most obvious applications is in the assessment of mixtures where some chemicals may act on different MIEs that contribute jointly to common downstream effects while others may have opposing effects on KEs pertaining to the same biological object (i.e., agonism and antagonism of the same receptor).

Inflammation is a common response to a variety of stressors, including xenobiotics. Under normal conditions, inflammation is a healthy and adaptive process that both combats infection and is involved in repairing damage to tissues. However, xenobiotics can elicit prolonged, severe, and/or inappropriate inflammatory responses that play a causal role in the progression of biological events linking a molecular initiating event (MIE) to an adverse outcome (AO). This is reflected in the observation that inflammation has long been known to play a prominent role in human disease and a variety of target organ toxicities.

Given its prominent role in target organ toxicities, it was anticipated that inflammation would emerge as a highly connected node within an AOP network. However, as inflammation is a complex, multi-stage process, it was not clear what would serve as the appropriate level of abstraction for describing one or more KEs that capture the most salient, measurable hallmarks of inflammation, while also accommodating linkage to the wide range of upstream causes and downstream effects that are associated with the process. In particular, there was no consensus on whether inflammation should be represented as a single KE, and if so, how it would be measured, or whether it should be divided into a series of KEs. Likewise, it was unclear whether the inflammatory process was unique to every tissue or whether there were common features that could reasonably be generalized across tissues. Finally, if inflammation was divided into more than one event, it was unclear whether those events would occur in sequence (i.e., following the linear construction typical of most AOPs), or whether the events would be so concurrent and inextricably linked that they would need to be represented as parallel or interconnecting branches or through introduction of an “and” type logic gate (i.e., A and B must happen in order to cause C).

Approach and Objectives

In order to address on-going uncertainties about how to best represent inflammation using the AOP framework, experts with different backgrounds in research, toxicology, and medicine, specialized in immunology, immune toxicology, cancer research, inflammatory diseases of different organs (liver, lung, kidney, brain) and endocrine-immune interactions convened at the Joint Research Center (Ispra, Italy), on 27–28 of September, 2017 to discuss

this challenge and provide some recommendations on how inflammatory processes might be represented using the AOP framework. Discussions focused on a number of key topics and questions. First and foremost was an emphasis on the biology and identifying the most salient and indicative features of inflammation. Inflammatory processes in different target organs were discussed and compared to identify the extent to which the process generalizes across various tissues and organs and what aspects of the biology are context-specific. The common understanding of the biology was then used as a foundation for discussion of an appropriate level of resolution/abstraction with which to describe inflammation using the AOP framework. Furthermore, important aspects of the quantitative understanding of KERs that underlie the process were considered. This included discussion of whether there were measurements or indicators that distinguish appropriate, healthy, inflammatory responses from adverse inflammatory responses. Likewise, the need to identify important modulating factors that are known to either influence the severity of an inflammatory response or alter an organism's susceptibility to develop an AO as a result of an inflammatory state (e.g., Roth et al., 2008; Maiuri et al., 2015) and therefore may need to be considered in inflammation-related KERs, was discussed.

The objective of the present manuscript is not to provide a comprehensive or critical review of the biology and toxicology of inflammation. Rather the aim is focused on representation of inflammatory processes using the AOP framework. Specifically, we sought to: (1) identify commonalities in the inflammatory process in different tissues and organs; (2) identify one or more KEs that could serve as common nodes in AOP networks; (3) agree on a consensus solution for integration of inflammation using the AOP framework; and (4) provide recommendations for how best to represent inflammation in the AOP framework and communicate these recommendations to relevant stakeholders (e.g., AOP developers; OECD; scientists studying inflammation). While it is expected that scientific uncertainty and debate over the specific details of inflammatory toxicology will continue, it is hoped that these recommendations provide a workable path forward for representation of this critical biology within the framework.

Salient Features of the Inflammatory Process

Regardless of the tissue, the inflammatory process can be organized into a number of sequential steps (Figure 1; Lawrence et al., 2002). Interaction of healthy tissues with a stressor can evoke the release of mediators that initiate inflammation. For example, direct damage to cells/tissues as a result of chemical reactivity leads to the release of molecular signals termed damage associated molecular pattern molecules [DAMPs], pathogen associated molecular pattern molecules (PAMPs, released by invading pathogens), or alarmins (Chan et al. 2012; Escamilla-Tilch et al. 2013; Schaefer 2014). These include a wide range of signal-initiating molecules that vary among cell types and include proteins, RNA or DNA, bacterially-derived lipopolysaccharides, small molecule metabolites (e.g., metabolic byproducts of bacteria, fungi, plants, etc.), and a variety of carbohydrates, which activate pattern recognition receptors, such as toll-like receptors and others. Certain xenobiotics may also mimic these molecular signals, without direct damage to cells. The tissue and stressor-specific combinations, concentrations, and durations of these signals is thought to "program" the ensuing inflammatory response.

The diverse upstream signals involved in induction of inflammation subsequently activate resident cells of affected tissues (Davies et al. 2013; Wynn et al. 2013). Activation refers to a phenotypic modification of the resident cells that includes alterations in their secretions, activation of biosynthetic pathways, production of pro-inflammatory proteins and lipids, changes in the metabolism and sensing of small molecules, and morphological changes (Hussel and Bell, 2014). While these represent a pleiotropic range of responses that can vary with the tissue, there are a number of common markers or signs of activation that are measurable.

Activation of tissue resident cells promotes the release of pro-inflammatory mediators, including pro-inflammatory cytokines, chemokines, vasoactive amines, and lipid mediators. Release of these signals into the tissue, as well as the circulation, promotes the recruitment of bone-marrow derived leukocytes (e.g., neutrophils and monocytes) that differentiate into mature pro-inflammatory cells, in response to mediators they encounter in the local tissue microenvironment. If persistent and unchecked, the pro-inflammatory actions of recruited cells can promote tissue injury, for example through the release of reactive oxygen and nitrogen species, which often characterizes acute adverse responses to pro-inflammatory signaling.

Under normal circumstances, once a sufficient pro-inflammatory response has been mounted, pro-inflammatory mediators will be supplanted by the release of anti-inflammatory mediators. These consist of a broad range of molecules that serve to both suppress immune response and promote resolution of inflammation and the repair of the damaged tissue. This phase is associated with differentiation of macrophages to an anti-inflammatory wound repair phenotype (Hussel and Bell, 2014). In cases where damage is on-going (chronic), leading to repeated cycles of damage and repair, fibrosis and other repair associated lesions can form in the affected tissue. These can eventually progress to the point of tissue dysfunction, triggering various diseases (AO). Conditions such as immune suppression, angiogenesis, and cell proliferation associated with repair can create favorable conditions for cancer formation. However, in cases where the damage is relatively acute and adequately repaired, the tissue can return to a “normal” homeostatic state, characterized as a generally quiescent cellular milieu.

It is important to note that although the inflammatory process can be organized into this generalized sequence, there is overlap between these states. Some signals ramp up as others decline. The cells in the tissue change dynamically in terms of both numbers and phenotypes as the process progresses. Additionally, waves of subsequent damage due to flare-ups of a pathogen, chronic exposures to a chemical stressor, or introduction of another insult (i.e., a “second hit”) can restart the process, such that there are overlapping waves occurring throughout different microenvironments in the tissue and organs that are not necessarily in phase with each other.

This all lends to a picture of inflammation as a highly complex process with stochastic components. While many of the actors, their roles, and the stages they act upon (in terms of tissue-specific milieus) are known, existing knowledge is generally not adequate to predict the specific company of actors that will respond to any particular stressor, their sequence, or

the specific outcomes that will ensue. The signals and cell types responding to any given stressor/exposure scenario are defined largely by the type and context of the tissue. Additionally, the factors deemed most relevant are influenced by investigator opinion and what those individual investigators choose to measure (or don't), which in turn shapes our current understanding of inflammation. While the same could be said for most fields of study, it is noted here in recognition of the daunting challenge we face in drawing quantitative or even semi-quantitative inference or predicting outcomes along inflammation-mediated AOPs. Although various markers or hallmarks of inflammation can reasonably be viewed as red flags or indicators of potential or even probable hazard, their application as reliable quantitative predictors of outcome is likely to be limited. With this caveat, we considered how inflammatory response can be represented and described using the AOP framework.

Hub Key Events for Inflammation-related AOP Networks

Through consideration of the salient features of the inflammatory process, three generalized KEs (tissue resident cell activation; increased pro-inflammatory mediators; leukocyte recruitment/activation) were proposed as candidates for common nodes (hubs) that could be shared by a diversity of AOPs involving inflammation (Figure 2). Each KE corresponds with one of the generalized sequential steps involved in inflammatory response in a tissue (gray shaded in Figure 1). Each of these broad processes was viewed as a generalizable hallmark of inflammation, independent of the tissue. Although they are closely linked, each can be measured independent of one another. Together, they are viewed as a minimum set of common denominators reflective in the inflammatory process across tissues.

Each of these events is consistent with the definition of a KE as reflecting a measureable change in biological state that is demonstrably essential to progression along the pathway, but not necessarily sufficient by itself to cause adversity (i.e., just because these events are observed, does not mean injury will occur – only that it *could*, based on current scientific understanding and evidence; Villeneuve et al. 2014a). However, these proposed hub KEs run counter to some of the conventions and best practices concerning KE description (Villeneuve et al. 2014b). Most notably, the title of the events does not clearly indicate what biological object (e.g., protein, metabolite, cell type, etc.) should be measured. This is because while the function/biological role/toxicodynamics of the event is well conserved across different tissues and organisms, the specific "actors" that are playing these roles, and thus how the measurements are made can vary. For example, in liver measures of "tissue resident cell activation" may focus on Kupffer cells, while in the brain they might focus on microglia or in bone osteoclasts (Davies et al. 2013). In terms of the ontological description of KE components (Ives et al. 2017), these KEs are viewed as well conserved in their "process" and "action" terms. However, the specific "object" terms can be expected to vary with tissue, environment and context and will need to be described for different tissue contexts as each KE description is expanded/revised for use with different AOPs (e.g., Table 1).

The three proposed hub KEs were viewed as reflecting functional points of convergence in relation to a diverse array of upstream, stressor-specific signals. Likewise, they were functional points of divergence in relation to different tissue- and context-specific outcomes.

Thus, they could be viewed as the “knot” in classic bow tie structures in systems biology (Friedlander et al. 2015) and thus are well suited to serve as central, highly connected, nodes within an AOP network (Knapen et al. 2017).

Pilot Implementation

Following the proposal of the hub KEs, corresponding event pages were created in the AOP-Wiki (https://aopwiki.org/events/1492; https://aopwiki.org/events/1493; https://aopwiki.org/events/1494). As a pilot, these events were integrated into a number of existing AOPs (Figure 3) linking different MIEs to distinct inflammation-mediated AOs or to AOPs where inflammation is an essential exacerbating element (https://aopwiki.org/; AOP 13; 38; 173; one novel AOP not yet in the AOP-Wiki). By introducing these hub KEs, it is now possible to link together AOPs that were previously disconnected (Figure 4) in the overall AOP network represented in the AOP-Wiki. The previously defined AOPs were readily adapted to incorporate the proposed hub KEs. For example, for AOP 38 (Figure 3C) Kupffer cell (resident macrophages) activation aligned with “tissue resident cell activation” and TGF-β1 expression corresponded with “Increased pro-inflammatory mediators”. The third hub KE “Leukocyte recruitment/ activation” had not been displayed as a separate KE in the original version of AOP 38, because that process had been incorporated into the description of the Kupffer cell activation KE. Likewise, in the case of an AOP leading to neurotoxicity (AOP 13; Figure 3B) it was possible to link neuroinflammation – which is considered a neurospecific process relying on specific neural cells - with the postulated hub KEs and their generic description. This allowed interconnectivity with other AOPs unrelated to the brain (Figure 4).

Next steps and Perspectives

Through deliberations on the biology and toxicology of inflammation, a consensus proposal was achieved with regard to an appropriate level of abstraction with which to represent the process of inflammation within a broad AOP network. The proposed level of abstraction resolves inflammation into three hallmark KEs that are expected to be functionally equivalent across multiple AOPs. However, the specific objects or actors involved in these functions are expected to vary by AOP according to the tissue specific context. This solves the primary challenge of representing inflammation in a way that is biologically appropriate yet is generalizable enough to facilitate AOP network connectivity, which was our major aim.

The approach recommended here is somewhat of a departure from current conventions regarding the description of KEs (Villeneuve et al. 2014b). While the biological function/ process each KE represents is well conserved across different tissue contexts, the specific objects (e.g., genes, proteins, metabolites) one would measure as appropriate indicators of these KEs are likely to be tissue-dependent. This will require creativity, careful thought, and perhaps some trial and error, in terms of how best to structure the KE descriptions for these events. Likewise, with experience, guidance regarding the minimum set of tissue-specific object changes that represent “triggering” of a given KE may need to be defined.

Nonetheless, it should facilitate our ability to represent inflammation in an AOP network context.

We and others (Leist et al. 2017) have noted the potential predictive limitations of inflammation-related AOPs based on the fact that they are inherently dependent on the relative balance of damage and repair and the associated timing and conditions of exposure. The proposed hub KEs do not specifically address that potential limitation to the predictive utility of the AOP framework in such circumstances. However, accepting this limitation, the ability to better represent inflammation in an AOP network is still highly beneficial. Specifically, it may help to more effectively differentiate those AOPs involving or intersecting with an inflammatory component, where more sophisticated models or in vivo testing may be required to predict outcomes, from those acting independent of inflammation where predictive confidence may be higher. In this way, the ability to identify intersection with inflammatory KE hubs aids the evaluation of fit-for-purpose of specific AOPs and AOP networks for different types of decision-making. Consequently, consensus around how to best represent inflammation via a set of hub KEs represents a significant step forward in the evolution and application of the AOP framework.

Acknowledgements

The authors thank R. Corvi, L. Gribaldo, T. Holley, S. Batista Leite, F. Madia, S. Munn, F. Pistollato, P. Prieto-Peraita, and M. Whelan of the JRC for contributing to discussions that led to this article. G. Ankley provided comments on an earlier version of this manuscript. Special thanks as well to E. Ahs for logistical and practical support. The contents of this manuscript neither represent, nor necessarily reflect the position or policy of the authors' institutions. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Funding information

This work was supported by the European Commission Joint Research Centre (JRC), Directorate F – Health, Consumers and Reference Materials and European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Ispra, Italy.

References

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* 29, 730–741. doi: 10.1002/etc.34. [PubMed: 20821501]

Chan JK, Roth J, Oppenheim JJ, Tracey KJ, Vogl T, Feldmann M, Horwood N, Nanchahal J (2012). Alarmins: awaiting a clinical response. *J. Clin. Invest.* 122, 2711–2719. doi: 10.1172/JCI62423. [PubMed: 22850880]

Davies LC, Jenkins SJ, Allen JE, Taylor PR (2013). Tissue-resident macrophages. Nat. Immunol 14, 986–995. doi: 10.1038/ni.2705. [PubMed: 24048120]

Edwards SW, Tan YM, Villeneuve DL, Meek ME, McQueen CA (2016). Adverse outcome pathways-organizing toxicological information to improve decision making. J. Pharmacol. Exp. Ther 356, 170–181. doi: 10.1124/jpet.115.228239. [PubMed: 26537250]

Escamilla-Tilch M, Filio-Rodríguez G, García-Rocha R, Mancilla-Herrera I, Mitchison NA, Ruiz-Pacheco JA, Sánchez-García FJ, Sandoval-Borrego D, Vázquez-Sánchez EA (2013). The interplay between pathogen-associated and danger-associated molecular patterns: an inflammatory code in cancer? *Immunol. Cell. Biol.* 91, 601–610. doi: 10.1038/icb.2013.58. [PubMed: 24100386]

Friedlander T, Mayo AE, Tlusty T, Alon U (2015). Evolution of bow-tie architectures in biology. PLoS. Comput. Biol 11:e1004055 [PubMed: 25798588]

Hussell T, Bell TJ (2014). Alveolar macrophages: plasticity in a tissue-specific context. Nat. Rev. Immunol 14, 81–93. doi: 10.1038/nri3600. [PubMed: 24445666]

Ives C, Campia I, Wang R-L, Wittwehr C, Edwards S (2017). Creating a structured AOP knowledgebase via ontology-based annotations. Appl. In Vitro Toxicol 3, 298–311. doi:.10.1089/aivt.2017.0017.

Kleinstreuer NC, Sullivan K, Allen D, Edwards S, Mendrick DL, Embry M, Matheson J, Rowlands JC, Munn S, Maull E, Casey W (2016). Adverse outcome pathways: from research to regulation scientific workshop report. *Regul. Toxicol. Pharmacol.* 76, 39–50. doi: 10.1016/j.yrtph.2016.01.007. [PubMed: 26774756]

Knapen D, Angrish M, Fortin M, Katsiadaki I, Leonard M, Margiotta-Casaluci L, Munn S, O'Brien J, Pollesch N, Smith C, et al. 2017 Adverse outcome pathway networks I: development and applications. (submitted October, 2017).

Lawrence T, Willoughby DA, Gilroy DW (2002). Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat. Rev. Immunol 2, 787–795. doi:10.1038/nri915. [PubMed: 12360216]

Leist M, Ghallab A, Graepel R, Marchan R, Hassan R, Bennekou SH, Limonciel A, Vinken M, Schildknecht S, Waldmann T, et al. 2017 Adverse outcome pathways: opportunities, limitations and open questions. *Arch. Toxicol.* 91, 3477–3505. doi: 10.1007/s00204-017-2045-3 [PubMed: 29051992]

Maiuri AR, Breier AB, Gora LF, Parkins RV, Ganey PE, Roth RA (2015). Cytotoxic synergy between cytokines and NSAIDs associated with idiosyncratic hepatotoxicity is driven by mitogen-activated protein kinases. *Toxicol. Sci.* 146, 265–280. doi: 10.1093/toxsci/kfv091. [PubMed: 25953702]

Roth RA, Luyendyk JP, Maddox JF, Ganey PE (2003). Inflammation and drug idiosyncrasy--is there a connection? *J. Pharmacol. Exp.* Ther. 307, 1–8. [PubMed: 12954806]

Schaefer L (2014). Complexity of danger: the diverse nature of damage-associated molecular patterns. J. Biol. Chem 289, 35237–35245. doi: 10.1074/jbc.R114.619304. [PubMed: 25391648]

Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, et al. (2014a). Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci 142, 312–320. [PubMed: 25466378]

Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, et al. (2014b). Adverse outcome pathway development II: best practices. Toxicol. Sci 142, 321–330. [PubMed: 25466379]

Wittwehr C, Aladjov H, Ankley G, Byrne HJ, de Knecht J, Heinzle E,, Klambauer G, Landesmann B, Luijten M, MacKay C, Maxwell G, Meek ME, Paini A, Perkins E, Sobanski T, Villeneuve D, Waters KM, Whelan M (2017). How adverse outcome pathways can aid the development and use of computational prediction models for regulatory toxicology. Toxicol. Sci 155, 326–336. doi: 10.1093/toxsci/kfw207. [PubMed: 27994170]

Wynn TA, Chawla A, Pollard JW (2013). Macrophage biology in development, homeostasis and disease. Nature. 496, 445–455. doi: 10.1038/nature12034. [PubMed: 23619691]

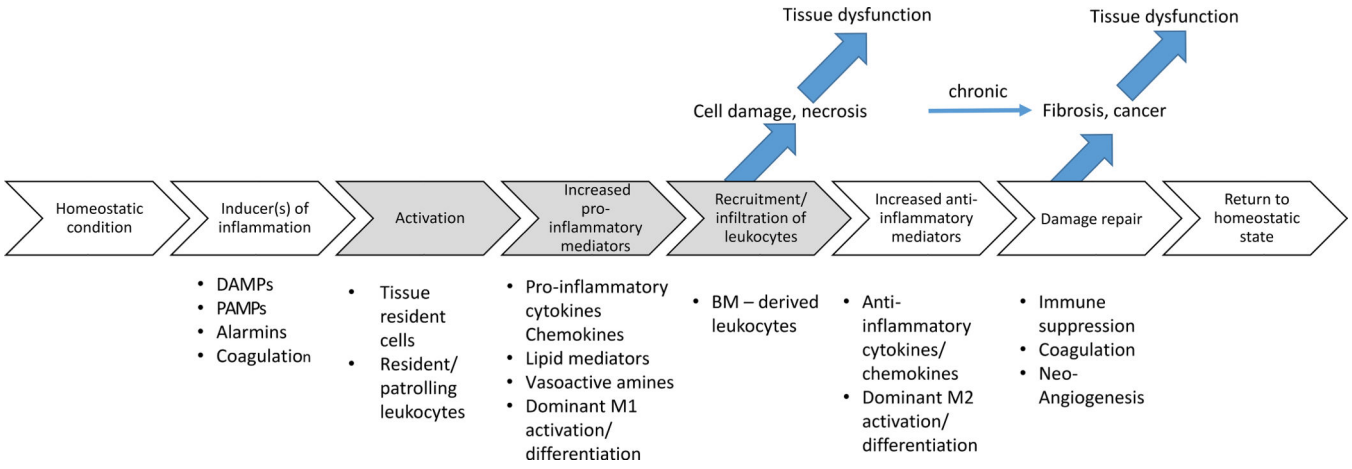

Figure 1.

Generalized model of sequential steps involved in inflammatory response in a tissue. Sequence of chevrons indicates anticipated adaptive/protective response to a damage signal. Arrows indicate trajectory diverging from an adaptive homeostatic state to a maladaptive/ adverse response. DAMPs = damage associated molecular patterns; PAMPs = pathogen associated molecular patterns; BM = bone marrow.

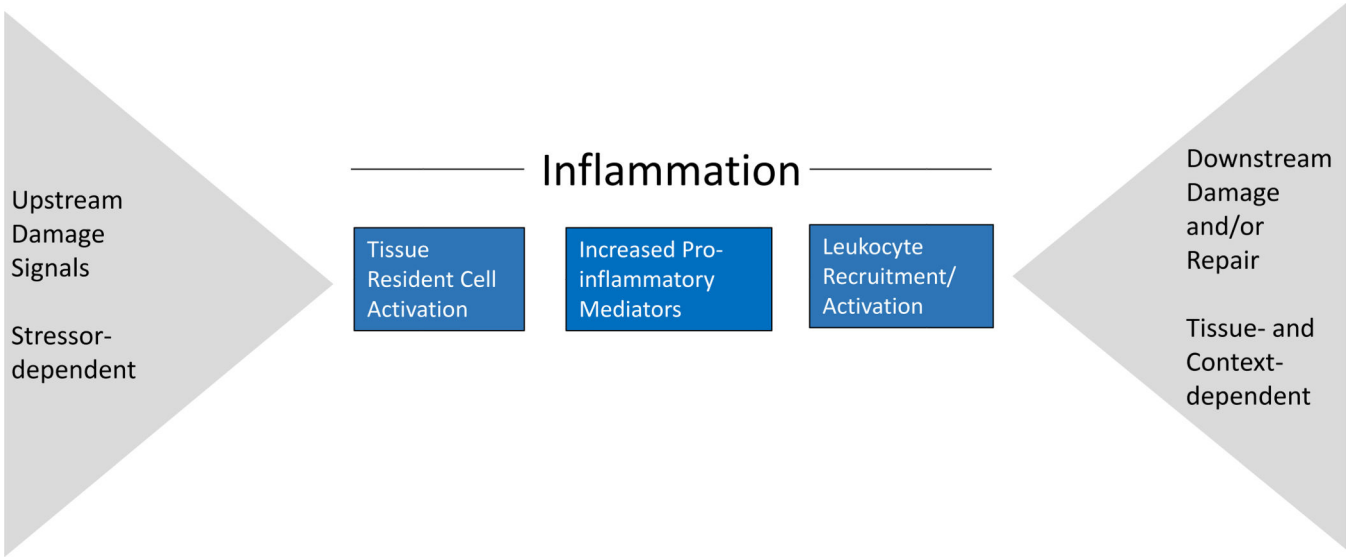

Figure 2.

Overview of three key events (KEs, blue boxes) associated with hallmarks of inflammation that were proposed for use as potential hub KEs in inflammation-related adverse outcome pathway (AOP) networks. The three events are viewed as points of convergence between a wide range of potential stressor-dependent upstream signals that can induce inflammatory response and points of divergence toward a wide range tissue and context-dependent adverse outcomes. One or all of these KEs may be included in a given AOP, and in some cases the order, particularly of the first two, may be reversed, consequently no arrows between the blue boxes are shown. Note “Tissue Resident Cell Activation” may also include activation of resident/patrolling leukocytes.

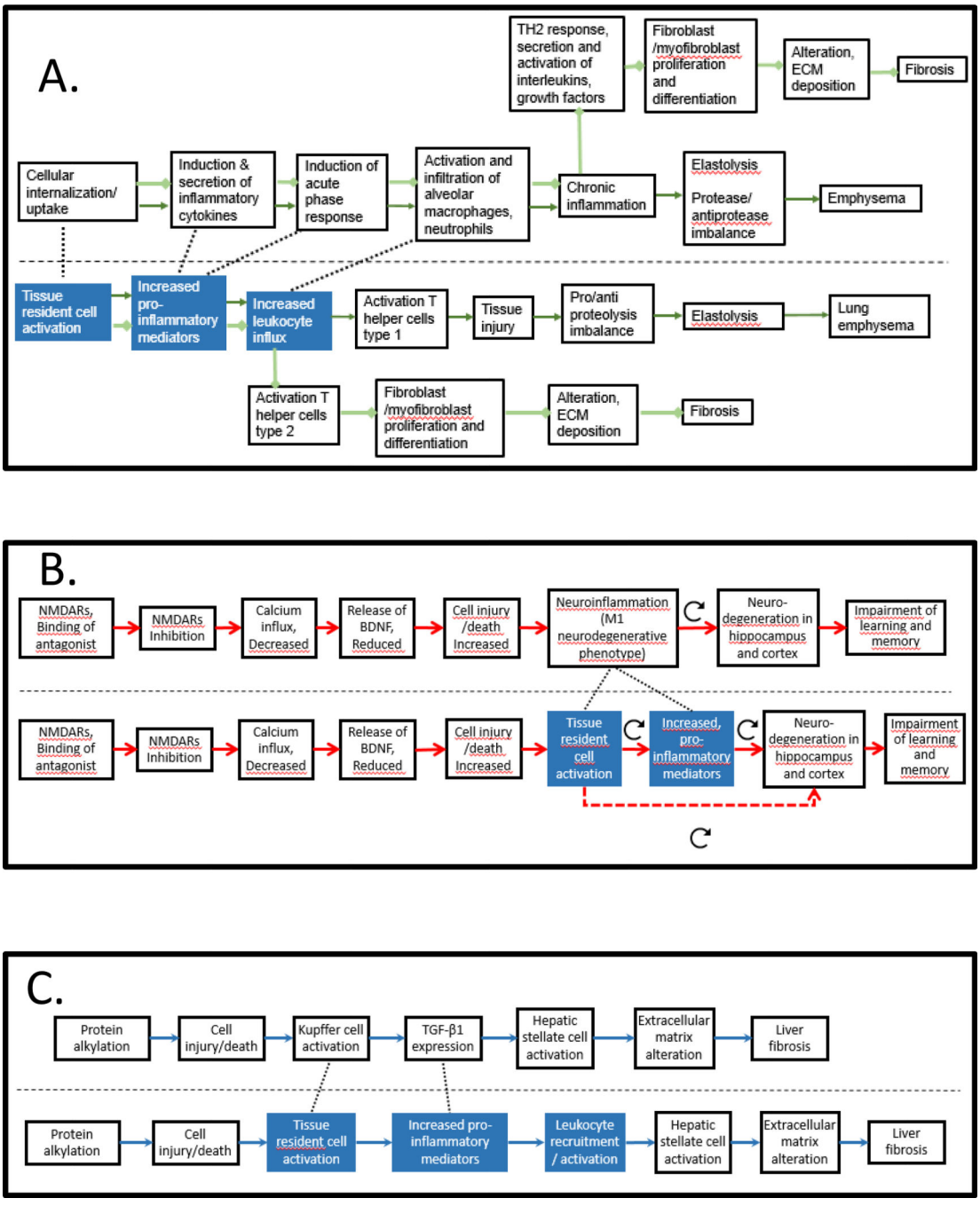

Figure 3.

Four adverse outcome pathways (AOPs) shown before (above the horizontal dashed line) and after (below the horizontal dashed line) incorporation of the proposed hub key events (blue shaded). A. A network of two AOPs linking induction and secretion of inflammatory cytokines to lung fibrosis (AOP 173; https://aopwiki.org/aops/173) or lung emphysema (novel AOP not yet entered in the AOP-Wiki). Revised titles, resident cell activation leading to lung fibrosis or lung emphysema, respectively. B. AOP linking chronic binding of antagonists to N-methyl-D-aspartate receptors (NMDARs) during brain development to

neurodegeneration with impairment of learning and memory in aging (AOP 13; https://aopwiki.org/aops/13). C. AOP linking protein alkylation to liver fibrosis (AOP 38; https://aopwiki.org/aops/38).

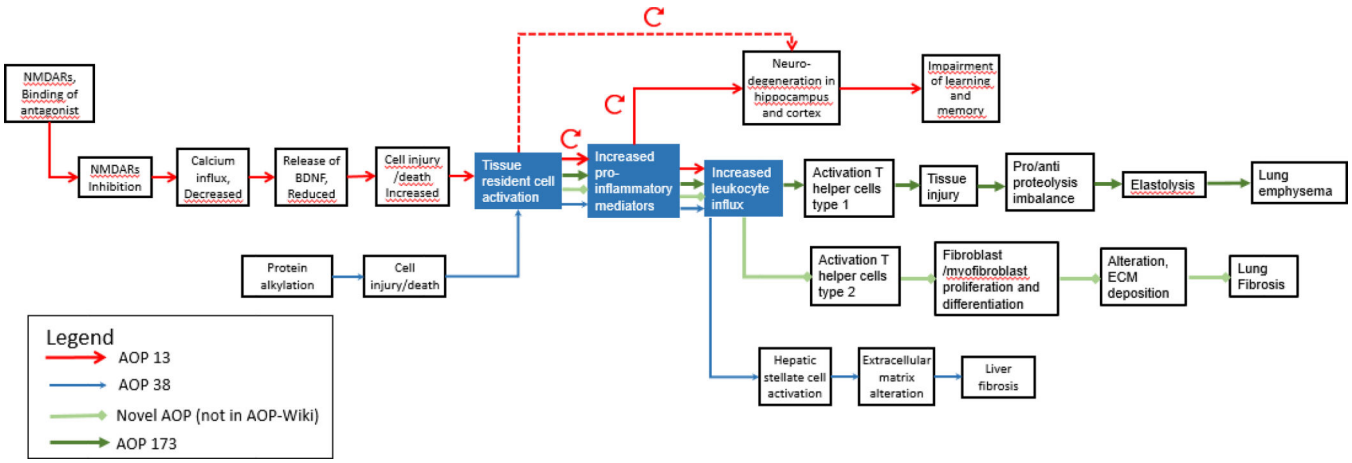

Figure 4.

Network composed of four adverse outcome pathways (AOPs; https://aopwiki.org/aops; AOPs 13, 38, 173 and one novel AOP not yet in the AOP-Wiki) illustrating how incorporation of the proposed hub key events facilitates improved connectivity of disparate inflammation-related AOPs within a broader AOP network.

Table 1.

Illustration of process/object/action terms (see Ives et al. 2017) that would define the proposed “hub” key event titled “increased pro-inflammatory mediators”. Note, the object listing is intended to be illustrative, not comprehensive.

Key Event Title	Event Components		
	Process	**Object**	**Action**
Increased pro-inflammatory mediators	Inflammation	TNF-alpha	Increase
		IL-1	
		IL-6	
		CXCL2	
		CCL2	
		Histamine	
		Prostaglandins	