Demystifying 'Danger': Conuersim between DAMPs and PAMPs

Abstract:

The current conundrum of inflammation is that exogenous Pattern Associated Molecular Patterns (PAMPs) from microbial sources or endogenous Damage Associated Molecular Patterns (DAMPs) released during trauma/tissue injury generate host Inflammatory response independently or in a synergistic manner. The proposed hypothesis suggests that they are interdependent and clinically inactive in isolation.

Introduction:

The existing paradigm on induction of Innate immunity and inflammation in mammalian physiology revolves around two classes of molecules – exogenous ones from microbial source, designated as Pathogen Associated Molecular Patterns (PAMPs) that are evolutionarily conserved in microbes and those of endogenous host origin called Damage Associated Molecular Patterns (DAMPs) released during tissue injury or trauma (1,2). Both the classes of molecules are largely perceived to use very similar set of pattern recognition receptors (PRRs) on immune cells and activate them leading to generation of innate immune activation characterized by release of host molecules that mediate inflammation (1-4). While there is broad consensus that activation of antigen presenting cells through Pattern Recognition receptors (PRRs) is crucial for induction of adaptive immunity in mammalian hosts (1,5), opinion is divided over the specific role played by DAMPs in mediating such cellular activation (6-10). Intuitively, immunologists find it convenient to accept PAMPs from microbial source as inducers of innate immunity that subsequently assist in generating adaptive immunity against the microbial immunogens. DAMPs on the other hand are being perceived as a set of 'mysterious' endogenous host molecules behaving functionally like PAMPs. Part of the skepticism is due to technical limitations of *in vitro* assay systems used widely to demonstrate cellular activation by DAMPs – traces of undetectable levels of PAMPs such as bacterial lipopolysaccharide (LPS) that are ubiquitously present in preparations of purified DAMPs could be contributing to the observed activation of cells in immunoassays (11,12). That chemically and structurally diverse sets of molecules such as DAMPs and PAMPs induce largely indistinguishable host responses in terms of their biological activity has further added to the mystery (13). The third and possibly more compelling reason for perceived skepticism

appears to be on the identity of receptors on host cells for PAMPs and DAMPs. The identity of specific germ line encoded host pattern recognition receptors (PRRs) for PAMPs of microbial origin have been identified by both structural as well as functional studies by experimentation *in vitro* and *in vivo* (14,15). However similar insights on identity of specific host receptors for the large array of DAMPs are not readily available. Functional studies suggest that DAMPs use receptors identified for PAMPs with a few notable exceptions (16) - P2X7 for extracellular ATP and RAGE for HMGB-1 have been identified to be the receptors by both functional and structural investigations (17,18). The most clearly recognizable and accepted difference between the two classes of molecules appears to be their cellular origin – DAMPs being endogenous 'self' molecules while PAMPs are exogenous 'non-self' molecules from microbial source. Historically, two models were proposed in the context of the roles by PAMPs or DAMPs in activation of 'Antigen Presenting Cells (APCs)' for initiation of adaptive immune response by the immune system. The 'Janeway' model emphasized the importance of microbial PAMPs, infectious non-self molecules that are recognized by germ line encoded specific pattern recognition receptors (PRRs) on antigen presenting cells (APCs) leading to their activation and release of inflammatory molecules essential for initiating adaptive immunity (19). The 'Matzinger' model proposed that the immune system primarily recognizes danger/damage to the host resulting in release of 'self' molecules as danger signals and it responds to such endogenous DAMPs by getting activated to release inflammatory molecules by APCs. (20) Both the models have been revisited and modestly revised over the years without substantially altering the fundamental differences (7-10,21). The above two models of activation of immune cells have been adapted by the inflammation community over the years to explain two types of host inflammatory responses in vivo – first, inflammation mediated by microbe or their products and the second, 'sterile inflammation' observed during trauma or tissue damage that involves only endogenously released host components in the absence of demonstrable pathogenic microbes. The two models viz., 'Janaway' and 'Matzinger' models have emerged as a bedrock for understanding infection associated inflammation induced by PAMPs and 'sterile Inflammation' mediated by DAMPs during the last 2-3 decades (22, 23). Evidence for synergy between the two molecules, PAMPs and DAMPs, have been reported extensively *in vitro* and modestly by *in vivo* studies although which of the two molecules functions as a primary stimulant and which as co-stimulatory molecule has been an issue for debate (23,24). While the role played by PAMPs in mediating infection associated inflammation has been widely accepted, apprehensions about the 'Danger model' has been sporadically discussed as mentioned above (8-10) - the major point of contention has been the

possible contamination of purified DAMPS with PAMPS (primarily ubiquitous molecule, LPS) resulting in the observed activation of immune cells *in vitro* 11-12). Meticulous attempts have been made to remove contamination of preparations of purified DAMPs with common PAMPs to establish the role of DAMPs to activate an inflammatory response. *Curiously however, the possibility of DAMPs contaminating in vitro culture systems (due to dying cells in vitro) to demonstrate PAMP mediated activation of cells has not been considered as a potential confounder by investigators!* The possibility that even nominal death of a very small percentage of cells (less than 0.5% of the cells releasing ng/ug concentrations of DAMPs) in *in vitro* cultures conducted over a period of 24-48 hrs contributing to contamination of culture systems with DAMPs such as extracellular ATP, HMGB-1 etc., have not been factored in while interpreting data on PAMP mediated activation of cells. Further, some of the DAMPs such as HMGB-1, HSP70 etc., display very high affinity for the ubiquitous PAMP, LPS. It is thus reasonable to assume that literature using *in vitro* activation of immune cells by PAMPs are vitiated by contamination of cultures with DAMPs. Thus, definitive conclusions on their sole ability to activate immune cells needs to be interpreted with caution since *in vitro* experimental artifacts could have contributed to the observations made in such in vitro experiments. Absence of sterile inflammation in mice deficient for PRRs such as TLR2 and TLR4 have been interpreted widely to imply that such receptors function as DAMP receptors (3,24). Purity of DAMP preparations such as HMGG-1 free of PAMPs has been a challenge to interpret these observations. In the absence of robust structural evidence, logic of PAMP receptors functioning as receptors for a large array of DAMPs appears weak currently. *In vivo* investigations on the other hand have offered more convincing evidence for synergy between PAMPs and DAMPs to potentiate inflammation. D-Galatosamine or Acetaminophen cause necrotic damage to liver and release DAMPs. Administration of sublethal doses of D-Galatosamine or Acetaminophen (that release DAMPs) and sublethal doses of LPS induce severe inflammation leading to mortality. Only D-Galatosamine or acetaminophen and only LPS at such doses induce low grade inflammatory response without mediating mortality of mice (25,26). Further, administration of extracellular ATP with LPS induced mortality of mice which could be blocked by quenching ATP by treatment with Apyrase (27)

Proposed Model:

In this 'perspective' the hypothesis being proposed is that PAMPs and DAMPs are interdependent molecules and do not act on host immune cells in isolation in the absence of the other. Essentially the model assumes that neither PAMPs nor DAMPs can activate host cells in the absence of the other. Cross talk between these two sets of structurally diverse

molecules leads to mutual amplification which determine severity of inflammation based on tissue/organ context and threshold of each of the molecules. Acute inflammation is primarily driven by microbial PAMPs supplemented by endogenous DAMPs and chronic inflammation is primarily driven by DAMPs supplemented by PAMPs. Like all models it is oversimplified but offers a working hypothesis supported by existing literature and allows for experimentation and validation.

Given that confounders of DAMPs being contaminated with PAMPs and the vice versa and experiments conducted *in vitro* cannot be relied upon with confidence to address the issue unambiguously, evidence for the current hypothesis has been sought only from limited sets of published data conducted in in vivo model systems. It is essential to recognize that extensive evidence for the existing dogma of cellular activation of immune cells by DAMPs and PAMPs leading inflammatory signals has been derived from experiments conducted *in vitro* using primary cells or cell lines. Similarly, identification of a growing number of DAMPs and their receptors using *in vitro* experiments also suffer from limitations of contamination with PAMPs. DAMP free in vivo model systems do not exist, even under physiological conditions, since basal levels of DAMP molecules such as HMGB-1, S100A9, OxLDL, Tenascin C, Hyaluronic acid, extracellular ATP etc are present in circulation and organs (28). Similarly, the commensal flora, primarily gut microbiota, contribute to presence of basal levels of PAMPs even under physiological conditions (29). In this context germ free mice can be considered as potential *in vivo* models for experimentation to address criticality of PAMPs in inducing sterile inflammation mediated by DAMPs to test the proposed hypothesis on interdependence between PAMPs and DAPMs in mediating inflammation. Available literature on induction of 'sterile inflammation' conducted in germ-free animals or in mice genetically deleted for specific genes/molecules involved in induction of inflammation has been used as evidence to support the proposed hypothesis to stitch together a unified model of host response by DAMPs and PAMPs (summarized in Fig 1 legend). Germ free mice are abnormal in terms of their immune system and physiology (30) and hence interpretation of induction of inflammation in such model systems need to be interpreted with caution. However, studies involving reconstitution with microbiota or a specific PAMPs such as LPS to recover the apparent deficiency of sterile inflammation offer confidence to use data generated in germ free animals as evidence for the hypothesis. Suggestive evidence for the proposed model of Interdependence between PAMPs and DAMPs from literature are summarized below (it is essential to note that the primary objectives of all these studies were not for demonstration of interdependence between PAMPs and DAMPs); a) Tissue damage leading to release of DAMPs has been found

to be a pre-requite for PAMP mediated activation in zebra fish model. Decoupling tissue damage mediated sterile inflammation and microbe induced activation allowed the investigators to conclude that in isolation the two fail to signal activation in the absence of the other (31). b) In a plant model of Arabidopsis the response to a PAMP, flagellin and a DAMP, plant elicitor peptide and their respective receptors led the authors to conclude that loss of function of either of the two receptors viz., FLS2 or PEPR resulted in impaired host response (32); c) direct evidence has been demonstrated for interdependence between DAMPs and PAMPs by non-canonical pathway leading to necroptosis in a mice model. Mandatory requirement of a PAMPs such as LPS along with a DAMP, HMGB-1 has been demonstrated for activation of Caspase-11and Gasdermin D resulting in necroptosis (33). Similarly, evidence for interdependence between LPS and another DAMP, extracellular ATP has been shown (34). Mice deficient in RAGE (receptor for HMGB-1) or P2X7 (receptor got ATP) were used to elegantly demonstrate activation of non-canonical pathway by LPS mediated along with DAMPs such viz., HMGB-1 and extracellular ATP (33,34), d) germ free mice were deficient in induction of zymosan mediated sterile inflammation which could be restored by treatment with LPS (35) and absence of carrageenan induced inflammatory pain in germ free mice could be restored by administration of LPS (36), f) acute inflammatory response mediated by monosodium urate was deficient in TLR-4 mice which was interpreted to mean that a DAMP like MSU signals thro TLR-4 by the authors while it could be due to dependence on endogenous LPS (37) while it could be interpreted to be dependent on presence of the endogenous PAMP, LPS; g) similarly, PAMP mediated inflammation has been found to be deficient in mice deficient in mice deficient for DAMP receptors such as RAGE, CD36 and P2X7 suggesting the need for DAMPs in PAMP induced activation of inflammatory responses (38,39).

According to the proposed model, under physiological conditions, normal immuno-competent animals exist with low levels of DAMPs and PAMPs and at such sub-threshold levels both the classes of molecules induce basal levels of inflammation that will be clinically insignificant. But pathological levels of host inflammation will be induced when the levels of one of them increases – PAMPs in the context of microbe induced inflammation and DAMPs in the context of ‘sterile inflammation’. The intensity or quantum of inflammation will be dependent on the threshold of the two class of molecules. Experimental evidence suggests that DAMP induced sterile inflammation is relatively weaker in comparison to microbial responses (40,41). In the event of tissue injury or trauma without demonstrable microbial intensity, the load of DAMPs is expected to be higher than levels of PAMPs contributed by microbiota. Similarly, during microbe mediated inflammation enhanced levels of PAMPs in the presence

of physiological levels of DAMPs from host due to constant recycling of cells will be involved. In the third scenario of tissue injury combined with microbial infection the levels of both PAMPs and DAMPs will be high resulting in high clinically and pathologically significant inflammation. This implies that blockade of either PAMP or DAMP could decrease inflammation and inhibition/neutralization of both will be far more clinically beneficial to the host. The salient feature of the proposed model is that PAMPs and DAMPs are to be treated as two independent entities activating independent pathways but as interdependent signals to induce clinically relevant inflammation - PAMPs activating PRR mediated activation pathway and DAMPs mediating inflammasome pathway and convergence of both being critical for amplifying in vivo inflammation (Fig 1). Acute inflammation is dominantly driven by microbial PAMPs complemented by physiological levels of DAMPs initially and gets amplified by tissue damage caused by the virulent microbial invasion - concentrations of physiological levels of PAMPs contributed by commensal microbiota are insufficient to cause clinical levels of inflammation. Chronic inflammation is dominantly driven by DAMPs and complemented by physiological levels of PAMPs contributed by commensal microbiota or by persistent latent infection with a microbe. The source of PAMPs can be either Pathogens or commensal microbiota and the immune system recognizes only the threshold of DAMPs and PAMPs and thus host inflammation is a balance between the two. The model is in concordance with earlier described basis of acute and chronic inflammation (42)

Demonstration of further experimental evidence for the proposed model could offer effective strategies for management of inflammation in humans and animals – inhibitors of PAMP pathway and inhibitors of DAMP pathway administered in combination can be expected to operate more effectively for management of inflammation respectively.

Way forward and validation of the hypothesis:

Existing limited literature on germ free mice and animals deficient for specific genes on pathways of immune activation do not provide unambiguous evidence to the proposed hypothesis. Germ free mice, which are completely free of PAMPs with decreased levels DAMPs of microbial origin, can serve as robust models for validating the hypothesis. Induction of sterile inflammation and/or activation of inflammasome pathway in the absence of NFkB pathway in such model systems after administration of DAMPs can be expected to offer clear verifiable evidence for the hypothesis. The most obvious translational consequence of the model will be that stimulation of innate immune response would need a blend of PAMPs and DAMPs and conversely inhibitors/ antagonists of both the pathways will more efficiently block acute as well as persistent inflammation.

The mandatory need for specific threshold levels of both PAMPs and DAMPs for generation of clinically demonstrable inflammation would also imply that neither the 'Janaway' model nor the 'Matzinger' model were wrong, but only that they were right by half! Historically, Ellie Metchnikoff's experiment with starfish in Messina, in which implantation of a rose thorn evoked vigorous macrophage reaction suggested to him about host immune defense. Ellie Metchnikoff may have missed the discovery of phagocyte activation if he had used a rose thorn in a germ-free starfish!

Note: *Conuersim in Latin means 'Interdependent;*
https://www.wordhippo.com/what-is/translations-for/interdependent.html

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References

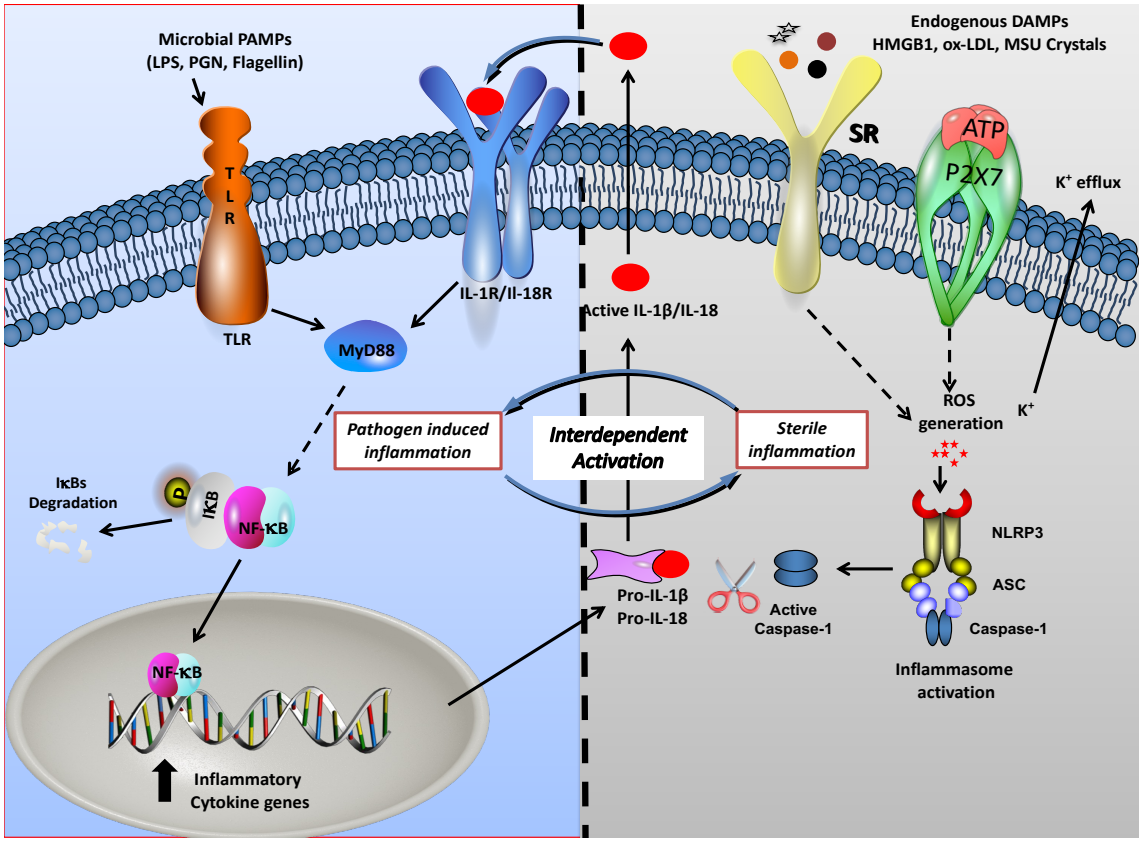
1. Pradeu, T, Thomma BPHJ, Girardin SE et al (2024) The conceptual foundations of innate immunity: Taking stock 30 years later Immunity *57*, 613-631 https://doi.org/10.1016/j.immuni.2024.03.007
2. Zindel J and Kubes P (2020) DAMPs, PAMPs, and LAMPs in Immunity and Sterile Inflammation Annu. Rev. Pathol. 15:493–518
3. Gong T, Liu L, Jiang W et al (2020) DAMP-sensing receptors in sterile inflammation and inflammatory diseases, Nat Rev Immunol volume 20 | February 2020
4. Danyang Li and Minghua Wu. Pattern recognition receptors in health and diseases Signal Transduction and Targeted Therapy (2021) 6:291 ; https://doi.org/10.1038/s41392-021-00687-0

5. Daolin Tang,Rui Kang, Coyne CB et al., (2012) PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol Rev 249(1): 158–175. doi: 10.1111/j.1600-065X.2012.01146
6. Jozefowski S The danger model: questioning an unconvincing theory Immunol Cell Biol 2016 Feb;94(2):164-8. doi: 10.1038/icb.2015.68.
7. Janeway C A, Goodnow C G and Medzhitov R Immunological tolerance: Danger — pathogen on the premises! Current Biology 1996, Vol 6 No 5:519–522 DOI:10.1016/S0960-9822(02)00531-6
8. Pradeu T and Cooper EL The danger theory:20 years later Frontiers in Immunolgy, 2012, 3, article 287. https://doi.org/10.3389/fimmu.2012.00287
9. Land WG, Messmer K 2012 The danger theory in view of the injury hypothesis: 20 years later Frontiers in Immunolgy, 2012, 3, article 349 https://doi.org/10.3389/fimmu.2012.00349
10. Cohn, M 2017 Giving context to Non-Self marker theories of Immune responsiveness, Scand J Immunol 86, 127-129
11. Tsan M-F and Gao B (2004) Endogenous ligands of Toll-like receptors, J Leuk Biol 76, 514
12. Tang D, Kang R, Zeh HJ et al (2023) The multifunctional protein HMGB1: 50 years of discovery, Nat Rev Immunol,23 824–841
13. Tang D, Kang R, Zeh HJ et al (2014) Complexity of Danger: The diverse nature of Damage-Associated Molecular Patterns, J Biol Chem 289, 35237
14. Takeuchi O and Akira S Pattern Recognition Receptors and Inflammation (2010) Cell, 140, 805–820, doi: 10.1016/j.cell.2010.01.022
15. Robinson JA and Moehle K, (2014), Structural aspects of molecular recognition in the immune system. Part II: Pattern recognition receptors, Pure Appl. Chem. 86(10): 1483–1538 https://doi.org/10.1515/pac-2013-1026
16. Gong T, Lei L, Jiang W and Zhou R (2020) DAMP sensing receptors in sterile inflammation and inflammatory diseases Nat Rev Immunol 20, 95-112 doi: 10.1038/s41577-019-0215-7
17. Di Virgilio F, Ben D D, Sarti A C, Giuliani A L, and Falzoni S. The P2X7 Receptor in Infection and Inflammation. Immunity 47, July 18, 2017 https://doi.org/10.1016/j.immuni.2017.06.020
18. Magna M and Pisetsky D S The Role of HMGB1 in the Pathogenesis of Inflammatory and Autoimmune Diseases, Mol Med 2014; 20(1): 138–146.

19. Janaway CA The immune system evolved to discriminate infectious nonself from noninfectious self, Immunology Today 1992, 13,11-16
20. Matzinger P (2002) The Danger Model: A renewed Sense of Self . Science 296, 301
21. Matzinger P The Danger model in its historical context Scand J Immunology 2011, 54, 4-9
22. Iyer S S, Pulskensc WP, Sadler JA, et al (2009), Proc Nat Acd Sci 106, 20388-20393 doi: 10.1073/pnas.0908698106
23. Huang Y, Jiang W and Zhou R, 2024, DAMP sensing and sterile inflammation: intracellular, intercellular and inter-organ pathways *Nat Rev Immunol* (2024). https://doi.org/10.1038/s41577-024-01027-3
24. Ma M, Jiang W and Zhou R 2024 DAMPs and DAMP-sensing receptors in inflammation and diseases Immunity, 57, 752- 771 https://doi.org/10.1016/j.immuni.2024.03.002
25. PK Barman, R Mukherjee, BK Prusty, S Suklabaidya, S Senapati and B Ravindran, 2016 Chitohexaose protects against acetaminophen-induced hepatotoxicity in mice 2016 Cell Death and Disease 7(5):e2224 DOI 10.1038/cddis.2016.131
26. Dong, W., Song, E. & Song, Y. Co-administration of lipopolysaccharide and D-galactosamine induces genotoxicity in mouse liver. *Sci Rep* **11**, 1733 (2021). https://doi.org/10.1038/s41598-021-81383-5
27. Cauwels A, Rogge, E, Vandendriessche, B, Shiva S and Brouckaert P (2014) Extracellular ATP drives systemic inflammation, tissue damage and mortality, Cell Death Dis. 2014 Mar; 5(3): e1102.
28. Roh JS, Sohn DH (2018) Damage-Associated Molecular Patterns in Inflammatory Diseases Immune Network 18(4):e27 https://doi.org/10.4110/in.2018.18.e27
29. Meessen ECM, Warmbrunn MV , Nieuwdorp M and Soeters MR 2019 Human Postprandial Nutrient Metabolism and Low-Grade Inflammation: A Narrative Review. Nutrients 11, 3000; doi:10.3390/nu11123000
30. Angela Wahl A, Yao W, Liao B et al (2024) A germ-free humanized mouse model shows the contribution of resident microbiota to human-specific pathogen infection Nature Biotechnology, 42 905–915
31. Huang C and Niethanmmer P 2018 Tissue damage signalling is a pre-requisite for Protective Neutrophil recruitment to microbial infection in Zebrafish, Immunity, 48, 1006-1013

32. Ma Y, Wlaker RK, Zhao Y et al (2017) Ligand linking perception by PEPR pattern recognition receptors to cytosolic Ca2+ elevation and downstream immune signalling in plants Proc Natl Acad Sciences (USA) 109, 19852- 19857
33. Deng M, Tang Y, Li W (2018) The Endotoxin Delivery Protein HMGB1 Mediates Caspase-11-Dependent Lethality in Sepsis, Immunity 49, 740–753
34. Yang D, He Y, Munoz-Planillo R Liu Q et al., 2015 Caspase-11 requires the Pannexin -1 Channel and the purinergic P2X7 pore to mediate Pyroptosis and endotoxic shock, Immunity, 43, 923-932
35. Karmarkar D and Rock KL 2013 Microbiota signalling throughMyD88 is necessary for a systemic neutrophilic inflammatory response, Immunol 140, 483-492
36. Amaral FA, Sachs D, Costa VV et al 2008, Commensal microbiota is fundamental for the development of Inflammatory pain Proc Natl Acd Sci (USA) 105,2193-2197
37. Rossato MF, Hoffmeister C, Trevisan G et al 2020 Monosodium Urate crystal Il-1b release is dependent on TLR 4 anf transient receptor potential V1 activation, Rheumatology, 59, 233-242
38. Nair, M., Jagadeeshan, S., Katselis, G. *et al.* Lipopolysaccharides induce a RAGE-mediated sensitization of sensory neurons and fluid hypersecretion in the upper airways. *Sci Rep* **11**, 8336 (2021). https://doi.org/10.1038/s41598-021-86069-6
39. Cao, D., Luo, J., Chen, D. *et al.* 2016 CD36 regulates lipopolysaccharide-induced signaling pathways and mediates the internalization of *Escherichia coli* in cooperation with TLR4 in goat mammary gland epithelial cells. *Sci Rep* **6**, 23132. https://doi.org/10.1038/srep23132
40. Bezbradica JS, Coll RC and Schroder K 2016 Sterile signals generate weaker and delayed macrophage NLRP3 inflammasome responses relative to microbial signals. Cellular & Molecular Immunology (2016) 13, 1–9
41. Eppensteiner J, Kwun J, Scheuermann U et al., 2019 Damage- and pathogen-associated molecular patterns play differential roles in late mortality after critical illness JCI Insight. 2019;4(16):e127925. https://doi.org/10.1172/jci.insight.127925
42. Furman, D., Campisi, J., Verdin, E. *et al.* Chronic inflammation in the etiology of disease across the life span. *Nat Med* **25**, 1822–1832 (2019). https://doi.org/10.1038/s41591-019-0675-0

Fig 1 Proposed Model:



Legend for Fig 1:

Model of Interdependence of PAMPs and DAMPS in induction of in vivo inflammation by canonical pathway:

Stimulation of Immune cells by PAMPs through PRRs such as TLRs, NLRs etc leads to activation of NFkB pathway. Concomitant stimulation by DAMPs thro Scavenger receptors, RAGE, P2X7 etc results in activation of Inflammasome pathway. Convergence of the two pathways is essential for generation and release of IL-1b and IL-18 that amplifies inflammation. Threshold of PAMPs and DAMPs will dictate differences in activation levels. The model assumes both PAMPs and DAMPs as mandatory requirements for activation and one of them in isolation will not be biologically active or clinically relevant. Presence of increased levels of DAMPs (during trauma/injury) in the absence PAMPs (as in germ free animals) will mediate defective induction of Sterile Inflammation. Generation of pathogen mediated inflammation results due to pathological levels of PAMPs in the presence of physiological levels of DAMPs. Similarly, sterile inflammation will be generated during trauma/injury that releases pathological levels of DAMPs in the presence of physiologically normal levels of PAMPs contributed by microbiota. Pathological increase of both PAMPs (by pathogenic microbes) and DAMPs (by high tissue damage) would result in induction of severe inflammation. The balance and threshold levels of PAMPs and DAMPs in an anatomical context will determine local or systemic inflammation. Activation of the two pathways by PAMPs and DAMPs are independent of each other, but their dependence on each other for synchronous activity will be critical for induction of clinically relevant inflammation *in vivo*.