‘Point if View’

Title: **Demystifying ‘Danger’: Conuersim between DAMPs and PAMPs**

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**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. This ‘opinion’ highlights several confounders in literature and argues in favour of addressing the issue only in *in vivo* model systems such as germ free animals that are free of microbiota and hence free of PAMPs in which response to DAMPs can be precisely studied. Based on available literature a model is proposed suggesting that host inflammatory response to PAMPs and DAMPs are interdependent and clinically inactive in isolation and that their thereshould and context would be critical factors in determining inflammation

**Short Summary:** Demystifying host inflammatory response to DAMPs

**Note:** *Conuersim in Latin means ‘Interdependent;*

***<https://www.wordhippo.com/what-is/translations-for/interdependent.html>***

**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). Both are largely perceived to use very similar set of pattern recognition receptors (PRRs) on immune cells for immune activation characterized by release of host molecules that mediate inflammation (1-3). 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, opinion is divided over the specific role played by DAMPs in mediating such cellular activation (4-6). Intuitively, immunologists find it convenient to accept PAMPs from microbial sourses 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 endogenous host molecules behaving functionally like PAMPs. That chemically and structurally diverse sets of molecules such as DAMPs and PAMPs induce largely indistinguishable biological activity is yet to be reconciled (7). PAMPs are recognised specifically by germ line encoded host pattern recognition receptors (PRRs) by both structural as well as functional studies (1-3). However similar insights on identity of specific host receptors for the large array of DAMPs are not readily available with a few exceptions such as ATP, HMGB-1, HSPs etc., and functional assays conducted in vitro. Some of the DAMPs like IL-33, HMGB-1 and S-100 proteins also classified as allarmins display duel cell function -inflammatory activation and play a role in wound healing (8). The primary confounder often proposed is the possible contamination of purified DAMPS with PAMPS (primarily ubiquitous molecule, LPS) resulting in the observed activation of immune cells (9). Curiously however, the possibility of DAMPs contaminating in vitro culture systems (due to dying cells releasing DAMPs) has not been factored in as a potential confounder for PAMP mediated activation by investigators. It is thus reasonable to assume that *in vitro* activation assays are vitiated by contamination by PAMPs as well as DAMPs and conclusions on their sole ability to activate immune cells need to be interpreted with caution. In the absence of robust structural evidence, proposals that PAMP receptors function as receptors for a large array of DAMPs appears weak currently.

**Proposed Model:**

**In this ‘viewpoint’ a 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. 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 determines severity of inflammation based on tissue/organ context and threshold 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 in 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. 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 (8). Similarly, the commensal flora, primarily gut microbiota, contribute to presence of basal levels of PAMPs even under physiological conditions. In this context germfree mice can be considered as potential *in vivo* model systems for experimentation to address the criticality of PAMPs in inducing sterile inflammation mediated by DAMPs to test the hypothesis on interdependence between PAMPs and DAPMs in mediating inflammation. Available literature on induction of ‘sterile inflammation’ conducted in germfree 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 1and its legend). Germ free mice are

abnormal in terms of their immune system and physiology 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 critical to note that the primary objectives

of all these studies were not directed towards demonstration of interdependence between PAMPs and DAMPs); a) Tissue damage leading to release of DAMPs has been found to be a prerequite for PAMP mediated activation in zebrafish 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 (10) 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 (11); currently 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 (12). d) germ free mice were deficient in induction of zymosan mediated sterile inflammation which could be restored by treatment with LPS (13) and similarly absence of carrageenan induced inflammatory pain in germ free mice could be

restored by administration of LPS (14), f) acute inflammatory response mediated by monosodium urate (MSU) 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 (15) 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 (16-17).

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 classes of molecules. 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. During microbe mediated inflammation enhanced levels of PAMPs in the presence of physiological levels of DAMPs 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. The salient feature of the proposed model is that PAMPs

and DAMPs are to be treated as two independent entities activating independent pathways but induce interdependent signals for clinically relevant inflammatory host

response - PAMPs activating PRR mediated activation pathway and DAMPs mediating inflammasome pathways and their convergence of respective pathways will be critical for 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. 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.

**Way forward and validation of 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. Historically, two models were proposed in the context of the roles by PAMPs or DAMPs in activation of immune cells (1,2) 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. 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. Both the models have been revisited and modestly revised over the years without substantially altering the fundamental differences (5,18). These 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*. 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. The mandatory need for specific threshold levels of both PAMPs and DAMPs for generation of clinically demonstrable inflammation as proposed in this ‘opinion’ implies that neither the ‘Janaway’ model nor the ‘Matzinger’ model were wrong, but only that both 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!

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**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*.