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INTRODUCTION

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Preformed mediators of defense—Gatekeepers enter the spotlight

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With every cut and bruise, every sore throat and runny nose, in all the numerous challenges our bodies face throughout life, our host defense systems spring into action and prevent escalation of the initial insults. They are essential for maintaining or re-establishing barrier function, for detecting and eliminating microbial intruders, and for restoring homeostasis. Higher species in particular, including humans, have developed elegant and efficient mechanisms that enable them to adapt to threats, raising a highly specific response and conferring immunological memory. Yet, the power of adaptive immunity may do little to protect us during a first encounter with new pathogens, and adaptive mechanisms need time to fully develop. For immediate and less discriminating activity against intruders and insults, our body, therefore, relies on host defense systems composed of preformed mediators that are readily available on cell surfaces and in the circulation. These first responders include pillars of innate immunity such as the complement, Toll-like receptor, and other pattern recognition systems; the cytokine network; the coagulation and contact systems; and antimicrobial peptides. Whereas many of these individual defense pathways have been known for a long time, it has become increasingly evident in recent years that they do not act in an isolated manner, but instead exert a high level of cooperativity and critically shape subsequent adaptive responses. Moreover, we now realize that the intrinsic ability to sense and react to non-self surfaces serves not only to eliminate pathogens but also contributes to other physiological surveillance functions, such as the clearance of cellular debris, tissue repair, and pruning during development. Even though the rapid and indiscriminate action of preformed defense mediators is carefully controlled under normal circumstances to prevent host-cell damage, these mechanisms sometimes become overwhelmed or misled, situations that may lead to a number of serious conditions (Figure 1).

With an emphasis on the human complement system and its intricate connections to other mechanisms, this special issue of *Immunological Reviews* on "Preformed Mediators of Defense" highlights the new perception of first-line host defense systems in health and disease. Thanks to the insightful contributions by opinion leaders and emerging scientists in their fields, the reviews in this issue cover a broad spectrum of topics and perspectives, ranging from evolutionary and clinical to deeply molecular aspects of host defense. Many of the systems involved originated from a common ancestor and became highly refined and specialized during evolution. The review by Elvington, Liszewski, and Atkinson retraces the fascinating evolutionary journey of complement from a primitive intracellular system largely relying on a single component (i.e. C3) to a multifactorial and well-connected protector of the interstitial space.[1] Interestingly, some of the ancestry appears to have been preserved in higher species, although it existed under the research radar for a many years; only recently have recycling and intracellular activation mechanisms been re-discovered.[1,2] In their review, Freeley, Kemper, and Le Friec delve deeper into this emerging aspect of complement function as a modulator of cellular homeostasis, discussing the differences between extra- and intracellular complement activity and highlighting its importance for T-cell induction/contraction and other crosstalk mechanisms.[2] Despite complement's functional diversification during evolution, C3 has remained the central component of the cascade that can initiate and amplify the complement response and is the node for the generation of most effector molecules. In our review, we summarize the accumulating insight into this versatile protein, discuss the molecular mechanisms that allow an abundant yet inert plasma protein to turn into a functional hub and orchestrator of immune responses, and illustrate the emerging roles of C3 as a trigger of disease conditions

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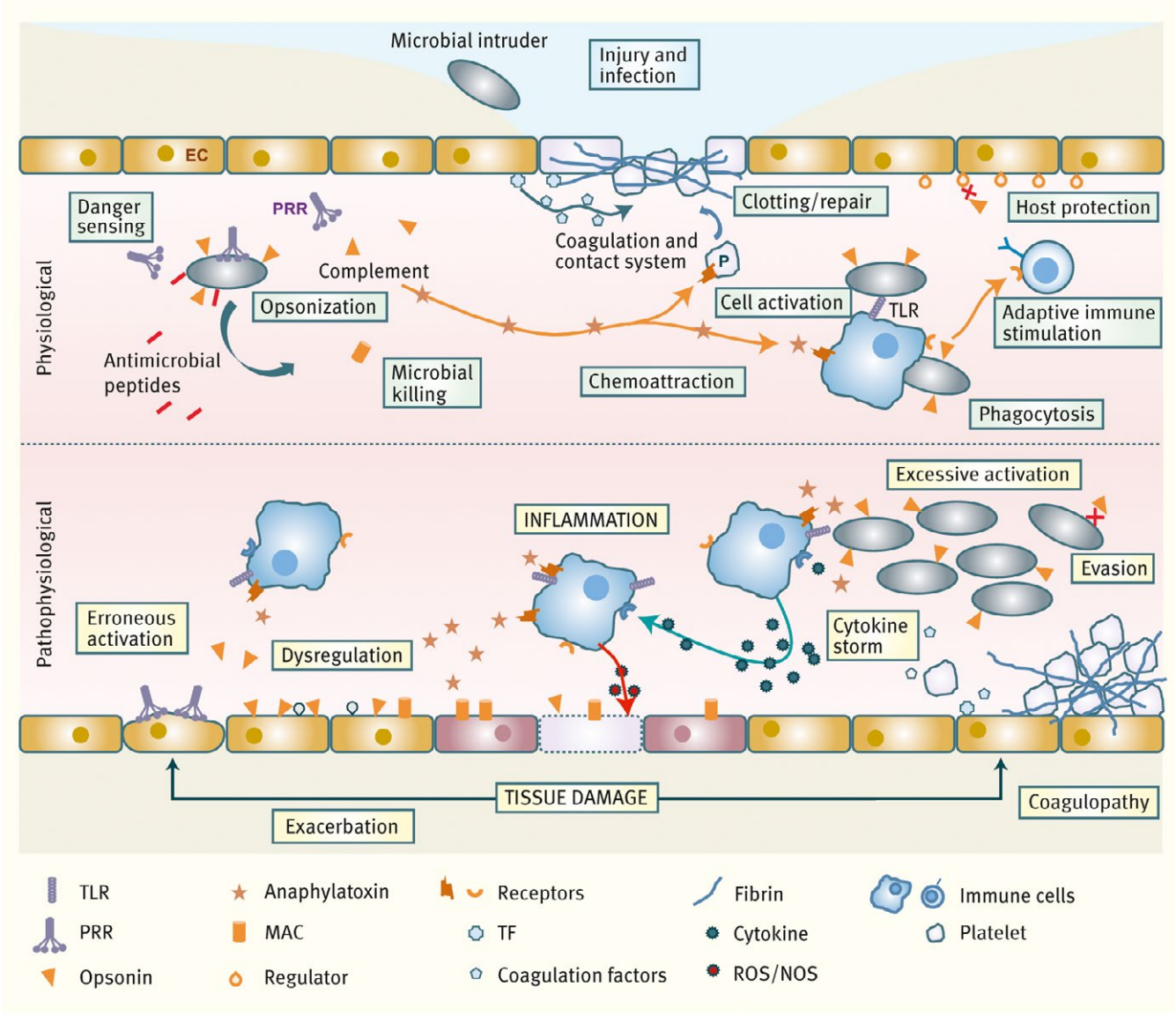

FIGURE 1 Preformed mediators of defense systems as double-edged sword in health and disease. Under physiological conditions, the various preformed defense mediators, including pattern recognition, complement, and coagulation/contact systems, act together to maintain barrier functions and clear pathogens after injury or infection (top). However, erroneous activation by diseased cells or massive triggering as during sepsis can lead to an overboarding hyperinflammatory and coagulopathic state that can exacerbate the damage and cause more harm to the body than the initial insult (bottom). MAC, membrane attack complex; NOS, reactive nitrogen species; PRR, pattern recognition receptor; ROS, reactive oxygen species; TF, tissue factor; TLR, Toll-like receptor

and as a therapeutic target.[3] The current picture of the inner workings of complement has been largely shaped by elegant structural studies, and high-resolution structures are now available for numerous complement components, effectors, and key functional complexes. The review by Schatz-Jakobsen, Pedersen, and Andersen takes us on a molecular journey from pattern recognition and proteolytic activation to the formation and regulation of the central C3 convertase complex, and the generation and function of C5-derived effector molecules.[4]

The sensing of altered and non-self cells is a defining function of complement and determines the initiation of particular activation routes. Whereas mannose-binding lectin has long been considered the sole initiator of the lectin pathway, several new pattern recognition molecules have recently been shown to contribute to the activation of this route, including ficolins and certain collectins. In their review, Garred and co-authors provide an overview of the well-known and emerging players in the lectin pathway; they also describe the formation, activation, and regulation of functional pattern recognition complexes and discuss the involvement of the lectin pathway in host defense and disease.[5] Alongside the pattern recognition molecules, the associated proteases (i.e. MASPs) constitute the key element of lectin pathway activity, although some aspects of these enzymes have for a long time remained elusive. The review by Dobo et al.[6]

illustrates the use of MASP-specific inhibitors for unraveling the distinct and common functions of MASP1-3 and their functional implications; together with a body of other research, such studies have revealed complex self- and cross-activating mechanisms, and shown an involvement of MASPs that reaches beyond the lectin pathway to encompass other complement routes, as well as coagulation and cell activation.[5,6] Once a complement response is initiated, the cascade can generate a series of potent effectors that lead to cell damage and facilitate clearance and/or act as attractants and activators of immune cells. Although these molecules have being described for decades, new and fascinating aspects of their functional spectrum keep emerging. For example, Verschoor and co-authors emphasize in their review that C3- and C5-derived effectors such as anaphylatoxins and opsonins are not only pro-inflammatory but are also immunomodulatory and function in close concert with other pathways. Mediating cell adherence and activation, these effectors may influence innate and adaptive immune responses under physiological conditions and in disease states such as asthma.[7] The interplay between opsonins and various complement receptors expressed by immune and other cells play a critical role in this context. With an emphasis on C3-derived opsonins, Erdei et al.[8] discuss our evolving understanding of the receptors' expression, ligand interaction and functional pattern. Membrane attack complexes (MAC), with their ability to kill microbes by forming lytic pores, are likely among the most well-known complement effectors. It is becoming increasingly evident, however, that the functions of the MAC and other terminal complement complexes extend well beyond bacterial killing, including activating and modulating effects. In their review, Morgan et al.[9] discuss the molecular mechanisms and functional implications of terminal complement complex formation and provide a summary of our structural knowledge about these potent effectors.

Given the potentially deleterious consequences of complement activation, a panel of soluble, surface-targeted and membrane-bound regulators carefully controls complement activity in circulation and on host cells, thereby protecting our bodies from self-attack. With a focus on the major regulator of complement amplification, i.e. Factor H, the review by Schmidt et al.[10] discusses individual regulators and common principles of complement regulation, their exploitation by microbial intruders, the clinical implications of complement dysregulation, and the potential of using regulatory molecules for therapeutic purposes. In contrast to the well-established negative regulation of complement on host tissues, the role of positive modulators is still emerging. Among these molecules, properdin has long been known as the stabilizer of convertase complexes, but as Blatt et al.[11] discuss in their review, this modulator likely has more versatile roles in shaping complement activation and the resulting thromboinflammatory responses than originally anticipated, and it may be considered a therapeutic target in some conditions. A comparatively recent discovery in complement modulation is that of the Factor H-related proteins (FHRs); their homology with the surface-recognition domains of Factor H appears to result in a competition for self-cell pattern and lead to de-regulation on certain surfaces. The review by Pickering and Medjeral-Thomas[12] provides a current overview of the various FHR proteins and their complexes, the genetic alterations that have been described, and the consequences of intact and disrupted de-regulation by FHR in a variety of complement-mediated diseases. Pentraxins are established pattern recognition molecules of innate immunity, but only recently has a close interaction been discovered between pentraxins, particularly PTX3, and complement. As highlighted by Daigo et al.,[13] the context-specific association of PTX3 with both complement activators and regulators is considered important for shaping the host response against microbes, cancer cells, and other threats, with potential clinical consequences and implications for diagnostics and therapy. Such close cooperativity between different preformed mediators, many of which were once considered members of unrelated pathways, in fine-tuning the host defense is now increasingly recognized and will likely lead to interesting future discoveries.

The interplay between positive and negative regulators, receptors, and effectors of the complement system becomes particularly important for the measured elimination of apoptotic host cells and other debris. In their review, Blom and Martin[14] provide an update on complement-mediated mechanisms that drive the safe clearance of apoptotic and necrotic cells, discuss an emerging concept of intracellular opsonization, and give a perspective on potential crosstalk mechanisms with inflammasome activity and other cellular processes. These insights add to a recent paradigm shift that comprehends complement and other preformed defense mediators not only as first-line responders but also as orchestrators of downstream immune and inflammatory processes that translate danger sensing into cellular responses via concerted receptor-mediated activation. As the review by Hajishengallis and Lambris[15] emphasizes, the cooperation and signaling crosstalk between complement and Toll-like receptors (TLR) appears to be of particular importance and can, depending on the context, have synergistic or antagonistic effects on the overall defense response; interestingly, the complement-TLR interaction is exploited by certain microbes to achieve dysbiosis or immune evasion that can contribute to periodontal disease and other inflammatory conditions.

Particularly strong crosstalk is observed between the evolutionarily related cascade systems of host defense, i.e. the complement, coagulation, contact, and fibrinolytic systems. The review by Ekdahl et al.[16] explores this sometimes helpful, sometimes devastating liaison between preformed mediators that is essential for maintaining barriers and defense but can also lead to thromboinflammatory complications if excessively or erroneously triggered; examples with clinical relevance range from transplantation and biomaterial-induced complications to thrombotic events during myocardial infarction or stroke. In their review, Alawieh and Tomlinson take ischemic stroke as an example to illustrate the impact of this unholy alliance between the complement, coagulation, and contact systems in a pathological context and highlight the therapeutic promise of using injury site-targeted complement inhibitors.[17] New insight has also been gained concerning the crosstalk between complement and the contact (kallikrein-kinin) system, as discussed in the review by Ghebrehiwet et al.[18]; mediated by the receptor gC1qR and involving other players,

this cooperation may assist host defense but has also been shown to have clinical implications, not only for hereditary angioedema but also for tumor progression and metastasis. The latter example underscores the dual role of defense pathways in cancer biology, which is the focus of the review by Berraondo et al.[19] While complement and other mediators may help to control tumor growth, most cancer cells possess mechanisms to escape attack by the host defense systems. Indeed, the generation of an inflammatory milieu in this situation may actually facilitate tumor progression. Conversely, complement, TLR, and other pathways may be exploited for therapeutic purposes in the fight against cancer.[19]

Of course, as in the case of the translation of immune responses, thromboinflammatory reactions are not restricted to soluble mediators but also rapidly induce cellular activity. In this context, the involvement of platelet and endothelial cells is of particular importance.[16,20] Roumenina et al.[20] emphasize in their review that endothelial cells are not just a passive barrier, but they also actively contribute to defense processes by secreting mediators, while at the same time adjusting their function on the basis of the activation profiles exerted by defense effectors. However, endothelial cells and platelets may also become targets and exacerbators of excessive defense processes, resulting in thrombotic microangiopathies such as atypical hemolytic uremic syndrome (aHUS) or contributing to transplant rejection.[16,20] Likely the most extreme examples of a defense response going awry are conditions related to systemic inflammatory response syndrome (SIRS). As showcased in the context of sepsis in the review by Delano and Ward[21], massive activation of host defense systems by microbial intruders, with a subsequent cytokine storm and immune cell activation, leads to a hyperinflammatory state that may cause more harm than the initial trigger, leading to thrombotic complications, tissue/organ damage, septic shock and, ultimately, death. The therapeutic modulation of defense responses in sepsis has proven to be challenging, but new approaches may provide novel avenues for preventing or taming hyperinflammation.

The new perception of preformed mediators of defense as a tightly connected and collaborating system that provides immune surveillance, orchestrates cellular responses, and helps maintain homeostasis has begun a new chapter in the exploration of host defense. At the same time, the realization that the same mechanisms that protect us may also cause harm and contribute to clinical conditions under other circumstances has brought host defense pathways into the spotlight of biomedical and pharmaceutical research efforts. Thus, it would not be surprising that the renewed interest in host defense mediators will reveal even more exciting connections and roles for these ancient pathways and may pave the way for new therapeutic options for numerous diseases.

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CONFLICTS OF INTEREST

The authors have no conflict of interest.

REFERENCES

1. Elvington M, Liszewski MK, Atkinson J. Evolution of the complement system: from defense of the single cell to guardian of the intravascular space. *Immunol Rev*. 2016;274:9–15.
2. Freeley S, Kemper C, Le Friec G. The 'Ins and Outs' of complement-driven immune responses. *Immunol Rev*. 2016;274:16–32.
3. Ricklin D, Reis E, Mastellos D, Gros P, Lambris J. Complement component C3 – The "Swiss Army Knife" of innate immunity and host defense. *Immunol Rev*. 2016;274:33–58.
4. Schatz-Jakobsen J, Pedersen D, Andersen G. Structural insight into proteolytic activation and regulation of the complement system. *Immunol Rev*. 2016;274:59–73.
5. Garred P, Genster N, Pilely K, et al. A journey through the lectin pathway of complement – MBL and beyond. *Immunol Rev*. 2016;274:74–97.
6. Dobo J, Pal G, Cervenak L, Gal P. The emerging roles of mannose-binding lectin-associated serine proteases (MASPs) in the lectin pathway of complement and beyond. *Immunol Rev*. 2016;274:98–111.
7. Verschoor A, Karsten C, Broadley S, Laumonnier Y, Köhl J. Old dogs – new tricks: immunoregulatory properties of C3 and C5 cleavage fragments. *Immunol Rev*. 2016;274:112–126.
8. Erdei A, Sándor N, Mácsik-Valent B, Lukácsi S, Kremlitzka M, Bajtay Z. The versatile functions of complement C3-derived ligands. *Immunol Rev*. 2016;274:127–140.
9. Morgan B, Walters D, Serna M, Bubeck D. Terminal complexes of the complement system: new structural insights and their relevance to function. *Immunol Rev*. 2016;274:141–151.
10. Schmidt C, Lambris J, Ricklin D. Protection of host cells by complement regulators. *Immunol Rev*. 2016;274:152–171.
11. Blatt A, Pathan S, Ferreira V. Properdin: a tightly regulated critical inflammatory modulator. *Immunol Rev*. 2016;274:172–190.
12. Pickering M, Medjeral-Thomas N. The complement factor H-related proteins. *Immunol Rev*. 2016;274:191–201.
13. Daigo K, Inforzato A, Barajon I, et al. Pentraxins in the activation and regulation of the innate immunity. *Immunol Rev*. 2016;274:202–217.
14. Blom A, Martin M. Complement in removal of the dead – balancing inflammation. *Immunol Rev*. 2016;274:218–232.
15. Hajishengallis G, Lambris J. More than complementing Tolls: complement–Toll-like receptor synergy and crosstalk in innate immunity and inflammation. *Immunol Rev*. 2016;274:233–244.
16. Ekdahl K, Teramura Y, Hamad O, et al. Dangerous liaison: complement, coagulation and kallikrein/kinin system crosstalk act as a linchpin in the event leading to thromboinflammation. *Immunol Rev*. 2016;274:245–269.
17. Alawieh A, Tomlinson S. Injury site-specific targeting of complement inhibitors for treating stroke. *Immunol Rev*. 2016;274:270–280.
18. Ghebrehiwet B, Kaplan A, Joseph K, Peerschke E. The complement and contact activation systems: partnership in pathogenesis beyond angioedema. *Immunol Rev*. 2016;274:281–289.
19. Berraondo P, Cordeiro L, Ajona D, Corrales L, Melero I, Pio R. Innate immune mediators in cancer: between defense and resistance. *Immunol Rev*. 2016;274:290–306.
20. Roumenina L, Rayes J, Frimat M, Fremeaux-Bacchi V. Endothelial cells: source, barrier and target of defensive mediators. *Immunol Rev*. 2016;274:307–329.
21. Delano M, Ward P. The immune system's role in sepsis progression, resolution and long-term outcome. *Immunol Rev*. 2016;274:330–353.