

Commentary

Toll-like receptors: the key to the stable door?

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Abstract

Severe sepsis continues to lead to critical illness. Few therapeutic options exist other than antibiotic therapy and general supportive care. Large numbers of patients continue to die as a consequence of overactivation of the host inflammatory response and the resultant coagulopathy and dysregulation of the normal controls of vasoactive tone. It is now known that a critical part of this host response occurs at the level of innate defence, without the need for antigen processing or the clonal expansion of cells targeted against the invading pathogen. This commentary will discuss the therapeutic targets revealed by our new understanding of the Toll-like receptor. The potential clinical difficulties that may result from intervention at this pattern-recognition receptor will also be explored.

Keywords innate immunity, intensive care, septic shock, Toll-like receptor

This commentary will discuss the clinical implications of the advances surrounding the Toll-like receptor (TLR), reviewed elsewhere in this issue by Opal and Huber [1]. Most human pathogens are readily killed *in vitro* by antibiotics. In addition, our antimicrobial therapy is so successful that it has provoked highly sophisticated adaptive changes by the pathogens themselves. Despite widespread use of these treatments, it has been estimated that there are 750,000 cases of severe sepsis per year in the US, at a cost of \$16.7 billion [2]. When there is progression to septic shock, mortality remains as high as 50% [2,3]. Indeed, despite over 30 randomised, blinded studies using blocking antibodies against inflammatory cytokines and their receptors [3], antibiotics remain the mainstay of treatment. Even recent successes in countering the derangement in coagulation and fibrinolysis are not consistent [4].

Critically, the marked variations in the tempo and intensity of the host response remain largely unexplained. We still do not know why some patients live and why some die. In meningococcal sepsis, fatal multi-organ failure can occur within a matter of hours despite appropriate antibiotic therapy and supportive care. Once a patient has developed such

derangement of their inflammatory cascades, our efforts in the intensive care unit are often akin to locking the stable door after the horse has bolted. It has taken a breakthrough in our understanding of how more simple organisms defend themselves to perhaps finally begin to offer the explanation as to why this might be.

Opal and Huber describe in their review [1] how the defensive mechanisms of the *Drosophila* fruit fly, which has no adaptive immunity, have been conserved to a remarkable degree in the innate immune response of higher mammals. The recent interest in innate immunity has been characterised by new understanding of several key components. These include antimicrobial polycationic peptides [5], bactericidal permeability-increasing protein, the triggering receptor expressed on myeloid cells TREM-1 [6], and macrophage migration inhibitory factor [7]. It is pattern recognition receptors on the host-pathogen interface, however, that represent one of the most exciting developments. Pattern recognition receptors, including CD14 [8] and CD11b/CD18 [9], recognise conserved regions on the invading organism called pattern-associated molecular patterns.

Opal and Huber review the recently described biology surrounding the latest member of the interleukin-1 receptor superfamily, the human homologue of the *Drosophila* Toll receptor, which was first described in Janeway's laboratory [10]. It appears that it is members of this TLR group that can differentiate potential pathogens (including viruses [11]) from self, using an apparently limited number of pattern recognition receptors. The rapid progress in our understanding of the TLR system [1] may soon allow us to answer some important clinical questions.

First, why can severe sepsis overwhelm a patient so quickly? Innate immunity operates at a very different tempo to that of the adaptive response since its effector cells perform their function without proliferation. For example, TLRs are not expressed in a clonal way: all such receptors displayed by cells of a given type, for example dendritic cells [1], have identical specificities.

Second, why do some patients live and some die? While the effect of Toll sequence polymorphisms on susceptibility to infection [12] may be as important as it is in plants [13], there may be other reasons for this interindividual variability. In endotoxin tolerance there appears to be important downregulation of TLR4 [14]. A failure of this mechanism could produce a devastating overactivation of inflammatory cascades. In addition, critical differences in host response could also result from variations in innate repertoire. The latter hypothesis is supported by the finding that the TLR system is far more complex than simply a collection of well-conserved germ-line receptors. This system is now known to involve soluble components and complex interaction or co-segregation of receptors; for example, RP105 and TLR4 heterodimers at the cell surface [15,16]. These cell-surface activities are linked to diverse intracellular events involving interrogation of pathogens at phagosomes by TLR2 [17] and activity of the intracellular proteins Nod1 and Nod2, which are structurally related to TLRs [16]. A final layer of complexity exists with various TLR pathways interacting at the level of transcription.

The cell surface collaboration between receptors is further complicated by the different receptor profiles presented by different host tissues. An important example of this is the finding that, under certain circumstances, gut epithelial cells [18] and hepatocytes [19] can both express TLRs. Both are key sites in the early events of septic shock and other severe sepsis syndromes like faecal peritonitis. In the latter, a large number of pattern-associated molecular patterns will flood the local TLR cell surface array. Indeed, mixed infection models do appear to produce adverse upregulation of the host response [20]. Beutler *et al.* have suggested that, while the host will tolerate some pattern-associated molecular profiles, others will trigger overactivation of inflammatory cascades [21].

Finally, how can we intervene in such crucial early events? Opal and Huber suggest three broad therapeutic strategies: the use of soluble TLRs specific for a particular organism; peptides or antibodies that interfere with extracellular domains of TLRs; or interference with intracellular events such as the recruitment of the adapter protein, MyD88. Such strategies will unfortunately suffer from the relatively late presentation of patients to the intensive care unit, thought to be one factor in the failure of most sepsis trials to date. Also, intervention may neutralise beneficial components of the host defence. In animal systems in which there has been successful prevention of lipopolysaccharide responsiveness with Toll knockout or blocking antibodies, the animals die from overwhelming bacterial sepsis [1]. Intervention may also block advantageous tolerance to subsequent fungal, bacterial or viral triggers.

In reality, progress in critical care derived from understanding of TLR biology may initially centre on improvements in established therapies. For example, the timing or mode of delivery of antimicrobial therapy together with other efforts to augment host defence [4] may turn out to be crucially important. The finding that TLR9 is sensitive to bacterial DNA [22], which is variably released before and after antibiotic therapy [23], may explain some of the adverse events that occur on using these agents [24]. The indiscriminate use of antimicrobials may also critically change the type of lipid A to which the host is exposed. Some lipid A species are recognised by TLR4 as an antagonist, while canonical lipid A from *Enterobacteriaceae*, a well-known group of noscomial organisms, would be seen as a TLR4 agonist [16]. Other more experimental interventions such as early high-volume haemodiafiltration or plasma exchange, which have a highly variable ability to remove both toxins and cytokines [25], could convert a protective downregulation to a harmful upregulation of host immune response.

In order that we can effectively intervene at the earliest events in the septic cascade, we may need to identify the ligands for TLRs, perhaps homologous to those found recently in *Drosophila* [26]. Description of the three-dimensional crystal structure of these crucial pattern-recognition receptors may also be required. Only then will we be able to claim that we have the key to the stable door.

Competing interests

None declared.

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