Sepsis under siege

A new understanding of sepsis might lead to the development of therapies to treat septic shock

The understanding of severe sepsis and septic shock could be on the verge of a breakthrough, in view of research overturning the long-held belief that the conditions are caused only by Gram-negative bacterial infections. The implications—and potential treatments—could be huge, as severe sepsis and septic shock affect 3 people in every 1,000 and kill at least 135,000 Europeans each year, about the same number of deaths caused by lung cancer. Treatment costs currently amount to about €7.6 billion in the EU alone, and existing therapies are able to save only a relatively small number of patients suffering from septic shock. Furthermore, the number of severe sepsis cases—over half of which end in death—is increasing at a rate of 1.5% per year as the number of immunocompromised people increases and the population ages.

Yet, despite these grim statistics, sepsis receives far less attention and media coverage than do other big killers, such as heart disease and cancer. This might be partly because severe sepsis is not really a disease, but rather a syndrome describing a systemic response. Invading pathogens release endotoxins into the bloodstream, which, in turn, activate various immunological and coagulatory responses that can go awry, leading to sepsis and septic shock. However, current research on the first stages of this immune response has led some researchers to propose that, in rare cases, sepsis can occur without an acute infection and in the absence of endotoxins.

The symptoms, risk factors and medical impacts of severe sepsis are clear. Clinical symptoms include high or dangerously low blood temperature, palpitations, cool blue

extremities and low blood pressure. In its most severe form, sepsis develops into acute septic shock as a result of an extreme fall in blood pressure, which starves the major organs and leads to characteristic symptoms, such as blackening of the extremities. In the absence of acute shock, prolonged sepsis can turn into a chronic disease that eventually leads to respiratory weakness and severe muscle wasting. In almost all cases, it impairs cognitive functions. Common risk factors include recent infection, burns or injury, surgery, diabetes, diseases that weaken the immune system—most notably AIDS—immunosuppressive therapies, for instance after organ transplantation or during cancer treatment, and old or very young age.

A growing consensus now contends that Toll-like receptors (TLRs), which have an important role in the first stages of infection by recognizing invading pathogens, also play a key role in developing sepsis. In particular, TLR4 provides the link between the prevalent theory that sepsis is triggered by endotoxins and the suggestion that it is caused by endogenous factors that might or might not be triggered by a bacterial infection. A pivotal moment came with the discovery of TLR and the sequencing of the human *TLR4* gene by Bruce Beutler and colleagues at the Scripps Research Institute (La Jolla, CA, USA) in the late 1990s (Poltorak *et al*, 1998). Further work revealed that TLR4 recognizes the endotoxin lipopolysaccharide (LPS) produced by Gram-negative bacteria (Poltorak *et al*, 2000). Since then, other TLRs have been identified that respond to different conserved molecular patterns, including viral as well as bacterial components. Some TLRs can recognize more than one component—indeed TLR4 responds to several structurally unrelated ligands including viral components as well as LPS.

In severe sepsis, TLRs wake up from their normal dormant state, which prevails in the absence of infection or other triggers, and cause the release of inflammatory mediators, including interleukin-1 and tumour necrosis factor (TNF). These in turn trigger local immune responses, such as vascular dilation, which lowers the blood pressure. At the same time, TLRs trigger the coagulation cascade, which under normal circumstances is a local response to restrict bleeding at the site of injury; however, in septic shock, this response becomes systemic and disseminates throughout the bloodstream. Because it inhibits fibrinolysis—the removal of fibrinogen clusters—it leads to the formation of micro-thrombi in the capillaries and eventually causes multiple organ failure.

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Before the discovery of TLRs, Kevin Tracey, from the Feinstein Institute for Medical Research (Manhasset, NY, USA), Beutler and others had already demonstrated that the immediate cause of septic shock was not the bacterial LPS but the excessive production of TNF by the host (Tracey *et al*, 1986). Until that point, the theory of sepsis had progressed little since Richard Pfeiffer's original discovery in 1892 that the severe toxicity of cholera was not caused by the *Vibrio cholerae* bacterium itself but by a poison released by the bacterium when it was destroyed by the host immune system (Pfeiffer, 1894). Pfeiffer's endotoxin theory was greeted initially with scepticism by some researchers, as was the 1986 discovery

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that the immune system has a causative role in acute septic shock. However, both quickly became standards in immunological theory.

The question now is whether a new theory, proposed by Jeffrey Platt and colleagues at the Mayo Clinic (Rochester, MN, USA; Brunn & Platt, 2006), will be accepted: that sepsis is caused by endogenous factors and can occur even in the absence of endotoxins. There is no dispute over the central role of TLRs, but the idea that these receptors can cause septic shock without any external trigger is more controversial. In a sense, it would represent another nail in the coffin of the idea that the immune system is always beneficial for the host. Researchers have long accepted that the immune system can act inappropriately, but this new theory of sepsis would mean that it could launch an attack on the organism it is supposed to protect even in the absence of obvious external agents.

Although autoimmune diseases have long been regarded as evidence of the failures of the immune system, it is still widely believed that such malfunctions have some underlying external trigger.

The causative role of endogenous factors was suggested in part by the continual failure of antibiotics to cure sepsis. "The most difficult thing to explain was how in trial after trial, if human patients infected by Gram-negative bacteria were treated by antibiotics or other drugs, there was no impact on the outcome of disease," said Platt. "They were neither better nor worse. It was almost as if endotoxin didn't matter." Platt concedes, though, that there are still problems with the idea that sepsis is caused by endogenous agents triggering TLRs. It begs the question of why sepsis does not occur more frequently, with the answer being, according to Platt, that TLRs are normally held in check. "So the first step in sepsis is release of the Toll-like receptors from suppression," he explained.

This explanation implies that something—be it internal agents or agonists—triggers the activation of TLRs. Cells expressing TLR4 do not normally activate an immune response in an environment that is rich in endogenous substances known to be effective TLR4 agonists, such as heparan sulphate, as their action is held in check by the extracellular matrix. However, when the matrix is degraded by proteases that cleave the connecting fibres, TLR4 inhibition is relieved, thus allowing the surrounding agonists to activate the receptor, according to Platt. So any event that delivers the relevant proteases can start the process, leading to sepsis. "These proteases get there at least in part through activation of the complement and coagulation systems," said Platt.

The Mayo researchers concluded that the first step in innate and adaptive immunity might be the release of TLR4 from its normal inhibited state by cleaving the extracellular matrix, a process further stimulated by

endogenous agonists, especially heparan sulphate. "The one common thing with all infections and injuries is some tissue damage, causing activation of complement and subsequent release of proteases," said Platt. "This, we think, is the seminal event in host defence and sepsis."

Although other researchers accept the important role of TLR4 in sepsis, they still insist that the activation must be triggered by endotoxins. "I do agree with Platt *et al* that host immunity in general and the Toll-like receptor 4 in particular play an important part in the pathogenesis of sepsis," said Anne-Cornelie de Pont, from the Academic Medical Centre at the University of Amsterdam, The Netherlands. "However I think the combination of an immunity disorder and a bacterial pathogen are necessary to develop the sepsis syndrome." Yet, Paul Knoebel, from the Division of Haematology and Blood Coagulation at the Medical University Vienna, Austria, believes that this new view would finally explain how systemic syndromes, such as sepsis, can arise in the absence of microbial infection. "Theories such as Platt's can explain why some patients develop a systemic, 'sepsis-like' syndrome not caused by microbial infection. Therefore, such aspects should be included in the current definitions of sepsis," he said.

Meanwhile, Tracey, one of the authors of the 1986 paper describing the role of TNF in sepsis, agrees that Platt's work is correct, but argues that it is just another step towards unravelling the molecular machinery that involves Toll-like receptors and their signalling. "Most people in the immunology world have known these receptors are important. I think that Bruce Beutler wouldn't have worked so hard on TLR4 if he hadn't appreciated its importance in sepsis," said Tracey.

There is a strong consensus around two key points—that current treatments for sepsis leave plenty to be desired, and that the Toll-like receptors, particularly TLR4, present promising drug targets in the future. Beutler himself believes that immunologists might well be on the verge of a revolution leading to a new generation of drugs capable of treating not only sepsis but also chronic inflammatory conditions and acute infections, because they all invoke the same inflammatory and coagulatory cascades. "In a chronic, sterile inflammatory disease like rheumatoid arthritis, TNF is clearly an important mediator," said Beutler. "The question then becomes, what is driving

production of TNF? If it is the TLRs, then probably they are the best targets to attack."

But such therapies are some way off. Beutler pointed out that it has yet to be proven beyond doubt that the biochemistry of both infectious and inflammatory diseases is similar. Until then it cannot be certain that drugs targeting TLRs will be effective against both. Meanwhile, according to Platt, the only therapy that has proven effective in the case of sepsis is the recombinant human activated protein C (rhAPC). "I think it inhibits the coagulatory cascade, and possibly other cascades responsible for releasing the Toll-like receptor from inhibition," he said. Indeed, many immunologists regard rhAPC treatment as the most important advance in the care of septic shock patients. The proof came in 2001 with the results of the worldwide PROWESS phase 3 clinical trial (Bernard *et al*, 2001)—it was halted early after rhAPC reduced mortality rates from 30.85% to 24.70%. Since then, there has been further progress unravelling the anti-coagulation mechanism of rhAPC and its interaction with other anti-coagulants, such as heparin and direct thrombin inhibitors. This correlates with the results of the recently completed XPRESS study, as yet unpublished, which demonstrated a reduction in mortality in a patient group treated with rhAPC and unfractionated heparin, compared with another group treated with rhAPC alone.

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RhAPC also helps to sustain blood circulation indirectly through its anti-inflammatory action. According to de Pont, it protects against cytokines by binding to protein C receptors in the endothelium of blood vessels, which helps to maintain microcirculation. However, rhAPC is only effective in a minority of cases and does not appear to relieve milder forms of sepsis. Attention has therefore turned to new drugs that directly target cytokines or the TLR4 receptor. One promising candidate is the high mobility group box protein 1 (HMGB1), which is also a constituent of the cell nucleus. HMGB1 can cause sepsis in mice, and work is in

progress to develop drugs on the basis of anti-HMGB1 antibodies, which block the progression of sepsis in animal models.

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But such drugs, even if they are more effective than rhAPC, are far from the end of the story. Even if the research community accepts the idea that the underlying causes of severe sepsis could be endogenous, it remains to be determined just what the external triggers are. It is obvious that TLR4 and cytokines, such as HMGB1, have significant roles, and that bacterial endotoxins are, of course, an important trigger. But non-infectious forms of sepsis require further research, as genetic factors are likely to have a part, together with cytokines that are normally constrained within cells. This too begs some questions, given that in chronic inflammatory conditions such as arthritis, tissue damage seems to be both a cause and a result of cytokine release. Just how the normal mechanisms of the immune response become disturbed in the first place is unclear. Deciphering this delicate balance promises to answer crucial questions not only in sepsis and septic shock but also in other diseases and disorders.

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doi:10.1038/sj.embor.7400742