

# The pathogenesis and molecular biology of sepsis

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The mammalian and microbial worlds exist in intimate proximity in a state of interdependence.<sup>1</sup> Their interactions can produce disease but — viewed through the larger prism of evolution — are also fundamental to the growth, development, and survival of complex species, such as humans. These interdependent processes cannot be properly understood in isolation, but rather are best viewed as a dialectical process in which sometimes the microorganism dominates, and at other times, the mammalian host.

This brief review does not aim to provide a comprehensive overview of an enormously complex topic, but will develop themes that are fundamental to the understanding and optimal management of sepsis:

- Host–microbial interactions are more commonly symbiotic than pathological.
- Tissue injury and disease are more a product of the response of the host than of the toxic activity of the bacterium.
- Harm results from a response to danger signals, which are both host and microbial in origin, and can be induced or aggravated by clinical intervention.

## Host–microbial interactions: symbiosis and pathogenesis

The world of eukaryotic multicellular organisms and the world of bacteria and other simple single-cell organisms are intimately entwined. The evolution of multicellular organisms more than a billion years ago was made possible when protobacteria invaded primitive single-cell organisms, establishing a symbiotic relationship and evolving to become the mitochondrion — the powerhouse of the cell.<sup>2</sup> The genome of the mitochondrion reflects its ancient origin as an independent bacterium. However, over time, genetic material has been shared between the mitochondrion and the nucleus, with the result that the vast majority of microbial genes now reside in the nucleus. About 22% of the human genome is made up of genes shared with bacteria, reflecting this common evolutionary history.

Other genes in the mammalian cell reflect invasion by viruses, whose protein products supported both viral persistence and the health of the host. For example, the cytokine interleukin-10 is a product of the Epstein–Barr virus that became incorporated into the human

genome.<sup>3</sup> A family of proteins termed inhibitor of apoptosis proteins are products of a baculovirus.<sup>4</sup> Virally infected cells can be identified and eliminated by host defences through the activation of apoptosis, or programmed cell death. Viral proteins that inhibited this process prolonged the survival of the virus, but also provided an evolutionary advantage in generating a further level of control over this process in eukaryotic cells. Indeed, multicellular organisms such as human beings are truly genetic mosaics, whose DNA reflects a long history of interaction and reconciliation with the microbial world.

Vestiges of this interaction exist within the cells, but an active interaction between mammalian and bacterial cells is a critical component of normal homeostasis. Human beings, for example, are made up of some  $10^{13}$  mammalian cells, comprising 250 different cell types. However, the mucosal surfaces of the normal healthy human being are populated with 10 times as many, or  $10^{14}$  bacterial cells,<sup>5</sup> representing between 500 and 1000 distinct bacterial species.<sup>6</sup> Bacterial genes outnumber human genes by a factor of three to one.

These interactions between the human host and the microbial world are fundamental to health and survival. For example, the bacterial flora of the gut plays a key role in gut development and function, and promotes the normal maturation of the human immune system.<sup>7</sup> Conversely, mice raised in germ-free conditions display multiple physiological abnormalities, including a significantly enhanced susceptibility to lethal infection with common microorganisms.<sup>8</sup>

However, microorganisms also pose a threat to the mammalian host. Exogenous organisms and organisms, such as the malaria parasite *Plasmodium*, or viruses such as HIV and the SARS virus, which are relative newcomers to human experience, can produce illness. Even the endogenous flora can produce disease when their relative proportions are disrupted, or when they breach normal mucosal barriers. As an extensive literature on microbial translocation shows, insults that alter the composition of the indigenous gastrointestinal flora can predispose to invasive infection with endogenous organisms.<sup>9</sup> The key, then, to optimising host defences against infection is not to eradicate all microorganisms, but to target specific pathogens, while supporting the normal homeostatic role of the endogenous flora.<sup>10</sup>

### Tissue injury is a consequence of the host response

Certain microorganisms produce toxins that directly target and injure host cells. For example, *Staphylococcus aureus* produces coagulase, which interacts with the coagulation cascade; streptococcal toxins function as superantigens; and the exotoxins of *Clostridium difficile* induce epithelial cell apoptosis. However, much more commonly, the tissue injury associated with invasive infection reflects the host response rather than the direct cytopathic effects of a microbial toxin.

The critical role of the host response in the pathogenesis of sepsis was established a quarter of a century ago, using mice with a genetic defect that renders them resistant to endotoxin. This mouse strain (designated C3H/HeJ) arose through a spontaneous point mutation in the parent strain (C3H/HeN). The parent strain was very susceptible to endotoxin, while the mutant offspring were highly resistant. Michalek and colleagues reported that susceptibility to endotoxin challenge could be transferred from sensitive to resistant mice by adoptive transfer of bone marrow cells.<sup>11</sup> In other words, the lethality of endotoxin challenge arose not from intrinsic properties of endotoxin, but from the fact that bone marrow cells of the host responded to endotoxin. Studies such as this laid the groundwork for elucidating the complex network of host-based inflammatory mediators that produces the clinical phenotype of sepsis. This has become the attractive, but still elusive, target of experimental therapies.<sup>12</sup>

Our research published in 1990 showed that it is the response of the critically ill patient, rather than the infectious insult, that causes the morbidity of critical illness.<sup>13</sup> In a cohort study of 200 critically ill surgical patients, we found that both the presence of infection (evidenced by objective microbiological criteria) and the degree of inflammatory response (measured by a sepsis score) predicted an adverse outcome, but that it was the latter host response that drove this risk. When patients with infection were stratified on the basis of this response, those who did not survive had significantly greater responses than survivors. Conversely, when patients with an activated response were stratified on the basis of microbiological variables, no such variables could be identified that predicted an adverse outcome. Even in this subset of patients, a greater inflammatory response was associated with a worse prognosis.

The recognition that sepsis derives from the response of the host, rather than the trigger that evoked it, has important and, as yet, only partially appreciated implica-

tions for clinical management. If it is the response of the host that produces disease, then there is no a-priori reason that it must arise from infection for a patient to benefit from therapies that modify the response. Indeed, given that the response is an adaptive process to eliminate a microbial threat, patients with active infection may be the worst population to target in randomised trials.

### The innate immune system recognises danger ... including the physician

Exogenous threats are recognised through cell-surface receptors encoded in the genome, which recognise “danger-associated molecular patterns” (DAMPs) that pose a threat to the host. The genetic defect in the C3H/HeJ mice proved to be in a cell-surface receptor known as toll-like receptor 4 (TLR4), which recognises endotoxin and permits the cell to respond to endotoxin in the cellular environment.<sup>14</sup> However, TLR4 is not specific for endotoxin, nor even for bacterial products. It can also be engaged, activating a classical inflammatory response, by host proteins such as elastase, heparan sulfate, and heat shock proteins.<sup>15</sup> Indeed, the triggers for activation of toll-like receptors and other receptors in the innate immune system appear to be primarily products of injured tissue.

Microorganisms can injure tissue; the host response to blunt trauma is qualitatively similar. However, the clinician can also cause injury, as a consequence of the interventions used to support the critically ill patient. Mechanical ventilation with low tidal volumes is known to improve survival;<sup>16</sup> conversely, mechanical ventilation with high tidal volumes not only results in a worse clinical outcome, but also activates inflammatory cascades reflected in increased levels of circulating inflammatory mediators.<sup>17,18</sup> Tissue ischaemia, resulting from either hypotension or regional hypoperfusion secondary to the use of vasopressors, is a similarly plausible insult that activates host inflammatory processes. Allogeneic blood transfusion can also activate danger responses. Finally, injudicious use of antibiotics can promote changes in mucosal colonisation in the gut that promote bacterial translocation and the absorption of endotoxin.

Thus, the phenotype of the complex clinical disorder of sepsis is initiated by the microorganism, sustained and amplified by the response of the host to tissue invasion, and ultimately further aggravated — albeit inadvertently — by the clinician’s attempts to correct the acute threat to life.

**Conclusions**

Sepsis is an inherently complex and conceptually inscrutable process. While we arbitrarily define the disorder as the host response to invasive infection, this gross oversimplification belies the fact that the innate immune system evolved not so much to identify microorganisms, as to permit the host to rapidly activate a protective response to danger in the environment. The endogenous microorganisms of the gut and mucosal surfaces can be a defence against this danger. The sterile consequences of tissue injury in, for example, pancreatitis may represent the response in its most full blown form. Our understanding of the dynamic interplay between insult, response, and the effects of clinical support is limited, and must be improved if we are to minimise the adverse sequelae.

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