Ignoring endotoxin

In 1947, Paul Beeson showed that rabbits repeatedly injected with certain bacteria eventually become resistant to the bacteria's fever-provoking effects—a phenomenon known as endotoxin tolerance.

When bacteria invade, the immune system reacts violently to bacterial endotoxin (or lipopolysaccharide [LPS])—a component of gram-negative bacterial cell walls. Why then does the immune system slowly stop reacting to LPS after repeated exposure? Perhaps this process allows the body to prevent excessive inflammation; with enough understanding, doctors may be able to manipulate the process too.

Endotoxin tolerance was first described in the early 1900s when fever therapy—the intravenous injection of fever-inducing bacteria LPS—was used in an attempt to shrink tumors and to treat certain infectious diseases (for review see reference 1). But patients often developed tolerance to the fever-inducing agent, requiring progressively higher doses to generate elevations in body temperature.

REsting blame

In the early 1940s, Beeson—then an assistant professor at Emory University—reproduced LPS tolerance in rabbits by injecting them daily with vaccine strains of bacteria. As in humans, the injections caused progressively milder and more transient fevers (2). Injecting endotoxin frequently was critical, as rabbits injected only once or twice per week developed only a low level of tolerance. The tolerizing effect was also nonspecific: tolerized rabbits remained fever free when exposed to either the original tolerizing strain or an unrelated strain of bacteria.

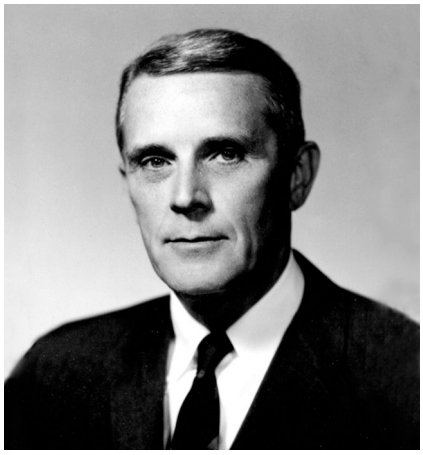
In search of a mechanistic explanation, Beeson attempted to transfer the effect using serum from tolerized rabbits. Rabbits that received the serum remained responsive to LPS, making it clear that antibodies to LPS were not sufficient. Also dispensable was the fever itself, as treating the rabbits with fever-reducing drugs did not block tolerance induction. The blame instead fell on cells of the reticuloendothelial (RE) system—now known to include macrophages and endothelial cells—as plugging up the RE system in tolerant animals (using a radiological contrasting agent called thorotrast) restored their responsiveness to LPS (3).

Beeson later showed that tolerance was associated with an accelerated clearance of LPS from the circulation. He hypothesized that repeated injections with fever-causing (pyrogenic) bacteria somehow enhanced the phagocytic capacity of RE cells, which rapidly cleared the bacterial toxin from the body, thus protecting other cells and tissues from its fever-inducing effects. Beeson published these observations in 1947 in *The Journal of Experimental Medicine* (2, 3).

Off the mark

Although later studies confirmed that endotoxin was cleared more rapidly from tolerant animals, Beeson's RE cell model did not hold up for long. "Beeson didn't do the right control experiment," says Sheldon Greisman, who studied endotoxin at the University of Maryland, "He didn't give thorotrast to nontolerant animals." When Greisman and his colleagues repeated the experiment, they found that the increased fever caused by blocking the RE system was seen in both tolerant and nontolerant animals—although the response was still milder in the tolerant animals (4).

Blocking the RE system, says Greisman, "was not simply diverting [more] endotoxin to other tissues." In fact, endotoxin primarily affected the liver, where the majority of RE cells reside. Later studies by Charles Dinarello (not using thorotrast) showed that liver macrophages from nontolerant rabbits produced a soluble pyrogen when stimulated with LPS; those from tolerant animals did not—a finding Greisman later confirmed in vivo (5, 6).



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Paul B. Beeson

Multifaceted mechanism

We now know that myriad mechanisms are involved in tolerance induction, including down-regulation of the LPS receptor Toll-like receptor (TLR)-4, decreased production of inflammatory cytokines, and alterations in TLR4-induced signaling pathways (for review see reference 7). Yet despite decades of research, the physiological role of endotoxin tolerance remains mysterious, perhaps in part because of an inability to inhibit tolerance induction without also eliminating initial LPS responsiveness.

But the evolutionary conservation of endotoxin tolerance suggests that it is a good thing. "If you've been bitten by a saber-toothed tiger," says sepsis specialist Mitchell Fink (University of Pittsburgh), "that is a big inflammatory stimulus, and you don't want to be consumed by systemic inflammation." But tolerance also has a down side: it occurs in critically ill patients, rendering them more susceptible to secondary infections. "If we can figure out how to manipulate this [phenomenon]," says Fink, "we'll have a way to manipulate the inflammatory response at will."

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