Sensing Endotoxins from Within

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at the data reveals huge bias, with diversity highest in the north temperate zones and most data points as birds. We know intuitively that biodiversity is not highest in Europe and the United States, and that most organisms are not birds. Thus, in addition to the robust debates about theory and methods that are part of today's biogeography, we must also pay attention to improving and adding to the underlying data and not neglect it in favor of studies that reuse the same data from single organism groups.

Today, the science of biogeography is more important than it ever has been, as concerns mount over the fate of biodiversity (*10*). As a comparative endeavor, it has the potential to shed light on how life and Earth have evolved together. But it is still necessary to heed Wallace's admonition: "None of these questions can be satisfactorily answered till we have the range of numerous species accurately determined" (*4*). Methods change and theories abound in this vibrant branch of science, but accurate data for comparison are critical for the holistic understanding of the relationships between Earth and its biota that biogeography seeks.

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IMMUNOLOGY

Sensing Endotoxins from Within

Macrophages respond to bacteria through a protein complex that promotes inflammation when activated by internalized bacterial endotoxin.

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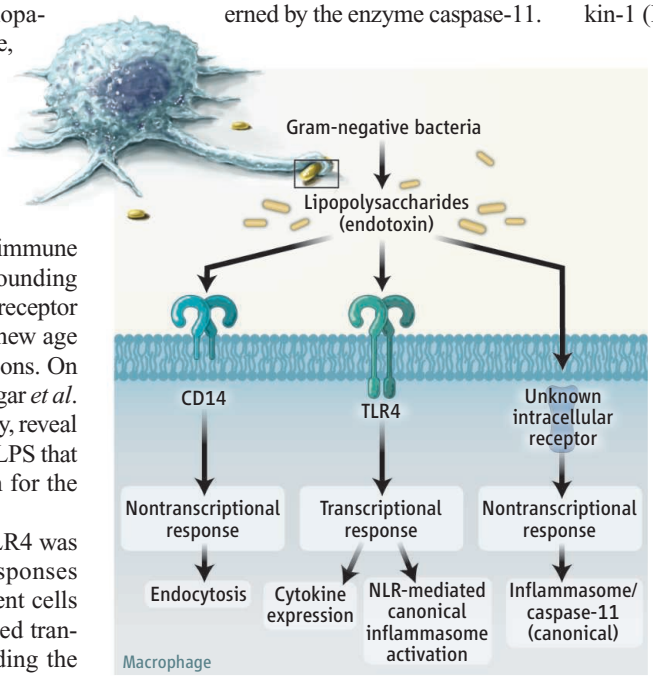
The human innate immune system identifies Gram-negative bacteria by recognizing lipopolysaccharides (LPS), components of the microbial cell wall (*1*). This detection triggers massive inflammatory responses that help eradicate infections, but may also result in immunopathology if regulated improperly. Hence, LPS is also referred to as endotoxin. More than a century after its discovery, the molecular basis for the inflammatory activity of endotoxin was finally revealed by the discovery that Toll-like receptor 4 (TLR4) induces innate and adaptive immune responses to LPS (*2*). TLR4 is the founding member of the mammalian Toll-like receptor family, and its discovery heralded a new age in the study of host-microbe interactions. On pages 1250 and 1246 of this issue, Hagar *et al.* (*3*) and Kayagaki *et al.* (*4*), respectively, reveal the existence of cellular responses to LPS that do not depend on TLR4. The search for the new LPS receptor can now begin.

For years, it was assumed that TLR4 was solely responsible for cellular responses induced by LPS (*5*, *6*). TLR4-deficient cells are defective for all classically defined transcriptional responses to LPS, including the expression of inflammatory cytokines and interferons (*7*). However, LPS can also induce nontranscriptional cellular responses, such as autophagy, endocytosis, phagocytosis, and oxidative bursts (*8*–*11*). Hagar *et al.* and Kayagaki *et al.* add to this list of atypical responses to LPS, by showing that LPS activates the formation of an atypical inflammasome governed by the enzyme caspase-11.



Sensing lipopolysaccharides. Macrophages respond to Gram-negative bacteria by activating transcriptional and nontranscriptional immune responses. Some of these responses are handled by protein complexes called inflammasomes. An intracellular sensor for lipopolysaccharides was revealed, but its identity has yet to be determined.

Inflammasomes are protein complexes that are assembled in the cytosol of macrophages in response to a variety of extracellular stimuli (*12*). The best-defined function of inflammasomes is to promote the processing and secretion of inflammatory cytokines of the interleukin-1 (IL-1) family. At the center of the best-characterized inflammasomes is the enzyme caspase-1, which cleaves the precursor of IL-1 in the cytosol of macrophages. Cleaved IL-1 family members are then secreted to induce inflammation. A second class of inflammasomes also requires caspase-11 to promote IL-1 cleavage (*13*). These noncanonical inflammasomes are activated by intracellular bacteria and contribute to the phenotypes associated with sepsis. How these noncanonical inflammasomes are activated remains unclear.

Hagar *et al.* and Kayagaki *et al.* recognized that several species of Gram-negative bacteria can activate caspase-11–dependent IL-1 secretion (*13*). Thus, a molecule common to Gram-negative bacteria must be responsible for activating caspase-11. Both research groups show that LPS is the molecule of interest. For example, Hagar *et al.* show that when transfected into macrophages, cell lysates derived from Gram-negative bacteria, but not Gram-positive bacteria (which contain no LPS), can

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activate caspase-11. This effect was insensitive to proteases and nucleases, thus ruling out the actions of any protein or DNA/RNA species in the bacterial lysate. However, the effect was lost when the lysate was treated with ammonium hydroxide, a chemical that inactivates LPS. Both Hagar *et al*. and Kayagaki *et al*. showed that transfection of pure LPS into macrophages was sufficient to activate caspase-11.

Interestingly, both Hagar *et al*. and Kayagaki *et al*. found that to activate this noncanonical inflammasome, LPS must be in a complex with transfection reagents, or some other means of delivering LPS into the intracellular compartment(s) of the cell. Because TLR4 senses LPS in the extracellular environment, this observation suggested that TLR4 was not responsible for activating caspase-11. Indeed, macrophages lacking TLR4 retained the ability to activate caspase-11 in response to transfected LPS. Thus, an additional LPS sensor must be present within cells. These studies provide the mandate to begin the search for this protein, and understand its function in host-microbe interactions and inflammation.

Although the identity of this new LPS sensor remains unknown, the studies by Hagar *et al*. and Kayagaki *et al*. provide some clues as to where this protein may reside in the cell. That LPS must be delivered into the cell suggests that the LPS sensor is present in an intracellular location. Hagar *et al*. showed that LPS can activate caspase-11 if it is in a complex with the Gram-positive bacteria *Listeria monocytogenes*, but only if the bacteria can enter the cytosol of the host cell. This indicates that the LPS sensor is a cytosolic protein. However, both studies also used the B subunit of cholera toxin as an "LPS delivery vehicle." Cholera toxin B follows a retrograde vesicular trafficking pathway from the plasma membrane to the lumen of the endoplasmic reticulum, but never accesses the cytosol (*14*). This raises the intriguing possibility that the LPS sensor does not reside in the cytosol, but instead in an intracellular compartment. Future genetic and cell biological studies are necessary to elucidate this fascinating new LPS sensory system.

The identification of a cellular response to LPS that does not require TLR4 draws parallels with recent work on other nontranscriptional responses to LPS. For example, the ability of LPS to induce endocytosis is controlled not by TLR4, but rather by the protein CD14 (*15*). Thus, a theme is emerging whereby TLR4 controls all transcriptional responses to LPS, but at least some of the equally important nontranscriptional responses are controlled by receptors other than TLR4 (see the figure). A future challenge will be to define not only the regulators of this new LPS response, but also how all responses to bacteria are interconnected in space and time.

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PHYSICS

Opportunities Knock with a More Stable Optical Clock

Improvements in the stability of atomic clocks could enable local gravity measurements and tests of fundamental physics.

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Recently, there has been rapid progress in the development of a new generation of optical atomic clocks that operate at frequencies around five orders of magnitude higher than their microwave predecessors. Because the instability of an atomic clock is inversely proportional to its operating frequency, optical clocks offer the prospect of achieving substantial improvements in measurement precision, potentially at the level of 1 part in 10^{18} for averaging times of a few hundred seconds. On page 1215 of this issue, Hinkley *et al*. (*1*) report an important step toward reaching this goal: a comparison between two ytterbium optical lattice clocks that demonstrates unprecedented clock instability of 1.6 parts in 10^{18} for an averaging time of 7 hours.

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Optical atomic clocks are based on ultrastable lasers that are stabilized to narrow atomic absorption features (clock transitions) in the optical region of the electromagnetic spectrum. Two different types of atomic reference are being studied as candidates for optical clocks, based on clock transitions in either single laser-cooled trapped ions (*2*) or thousands of laser-cooled atoms trapped in an optical lattice (*3*). The optical lattice type is advantageous with respect to theoretically achievable instability, because a larger number of atoms yields an improved signal-to-noise ratio in the measurement of the atomic absorption frequency.

Despite this theoretical advantage, the demonstrated stability of optical lattice clocks was, until recently, similar to that of optical clocks based on single ions, mainly because of technical limitations imposed by the noise of the clock laser used to probe the narrow absorption feature. Because a measurement cycle includes time for laser cooling and state preparation of the atoms, the difference between the clock laser frequency and the atomic absorption frequency is measured only during a fraction of the complete cycle time. This periodic sampling of the clock laser noise degrades the achievable stability of the clock, a phenomenon known as the Dick effect (*4*).

When comparing two optical lattice clocks, the Dick effect can be eliminated by probing the two clock transitions synchronously with the same clock laser (*5*). Although this procedure can be useful in reducing the averaging times required to evaluate the performance of the clock, it does not improve the instability of each individual clock as a timekeeping device. Other approaches to minimizing the Dick effect, such as reducing the instability of the clock laser and minimizing the dead time in the measurement cycle, are required. Key to the new results reported by Hinkley *et al*. is their