In the 1700s, it was discovered that protein extracts from rotting or putrid materials could produce a fever in human and animals [1]. Over the next two centuries, many researchers used protein extracts of various sources, milk being a favorite, and verified these observations. As reproducibility was an issue, Professor Peter Pannum performed a series of methodical and systematic experiments in the 1870s to characterize the properties of this mysterious pyrogen. It would precipitate with protein but was not itself a protein, was soluble in water but not alcohol, and was extremely heat-stable [1]. The issue of reproducibility was explained when it was found that this “endotoxin” was often found in the supposedly sterile water or saline used for these experiments due to the presence of bacteria and specifically, Gram-negative bacteria. In fact, Florence Seibert demonstrated that even aseptically collected cow’s milk, which was a favorite protein source to induce this response, contained viable Gram-negative bacilli, which was quite probably the first description of what we now call LPS. Then, as now, LPS contamination or the threat thereof has led to heated debates in the interpretation of experimental results [3]. Although differences in LPS responsiveness between and within species were recognized before Seibert’s time, this was attributed to methodological or anatomical differences, since the contribution of host genetics was not understood. We now know that LPS responsiveness varies significantly within populations of humans and between strains of mice as a result of genetic factors that influence LPS signaling directly and indirectly (e.g., through modulation of the cytokine microenvironment). In this issue of the JLB, Raza and colleagues [4] tackle the complexities of the LPS response by comparing the transcriptional profiles of bone marrow-derived macrophages from two strains of mice, C57BL/6 and BALB/c, stimulated with a range of doses of LPS in the presence and absence of type I and II IFNs.

The inflammatory response is potent and metabolically costly, and the adjustment of the magnitude of inflammation to the size of the threat is essential to prevent immunopathology. By investigating the role of dose and the kinetics of the LPS response, the authors are able to quantitate transcriptional thresholds of responsiveness. The authors found that although the majority of the genes (56%) altered by LPS increased in a dose-dependent manner, 25% were maximally expressed at the lowest dose, and almost 10% demonstrated decreased expression with an increasing dose of LPS (Fig. 1). These data are consistent with experimental observations that LPS responses and indeed, many infectious disease models, use fundamentally different inflammatory pathways based on the magnitude of the challenge [5]. Differences in the threshold of responsiveness are reminiscent of the human condition, where polymorphisms in TLR4 or downstream adaptor molecules have been shown to alter responsiveness to LPS. Those associated with decreased responsiveness may be linked to increased sensitivity to infectious disease, sepsis, and inflammatory bowel disease but may be protective against cardiovascular disease and atherosclerosis [6]. The fact that as many of 10% of the population have naturally occurring polymorphisms within TLR4 demonstrates that there is evolutionary pressure to maintain those who are more sensitive and those who are more resistant [7]. Although this natural variation is lost in our mouse models, the differences in gene expression between low and high doses of LPS are a reminder of how many aspects of the LPS response are calibrated to the magnitude of stimulation.

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Figure 1. Raza et al. [4] characterize a number of transcriptional responses of macrophages responding to LPS, IFN-γ, and IFN-β separately and in combination. They find that there are (I) LPS-dependent responses (which may include responses to autocrine IFN-β), (II) LPS-independent but IFN-γ- or IFN-β-dependent, in addition to (III) common transcriptional responses to all three stimuli. LBP, LPS-binding protein; MD2, myeloid differentiation protein 2; TRIF, Toll/IL-1R-domain-containing adapter-inducing IFN-β; TRAM, TRIF-related adaptor molecule; IFNAR, IFN-γ receptor; GO, Gene Ontology.

The authors have previously compared transcriptional profiles to LPS among humans, mice, and other species and found considerable differences between species and mouse strains [8]. Herein, they confirm that there are transcriptional differences between the two strains in the steady state and upon LPS stimulation. Interestingly, some genes expressed in one strain are completely absent in the other, and many of these differences lie in well-studied pathways, such as the complement pathway, antigen presentation, and inflammatory cytokines. The genetic variation observed between the two mouse strains demonstrates how divergent these strains have become and how problematic generalizing the mechanics of the inflammatory response, based on studies of one strain of inbred mouse, is. Although some have called into the question whether mice are a useful model for any studying any aspect of the inflammatory response [9], some seemingly divergent responses between species and experiments may be the effect of dose, source of macrophages, or culture conditions rather than species per se.

The authors use bone marrow-derived macrophages, which are the macrophage biologist’s favorite model for their numbers and consistency; however, they are themselves a proxy for fully differentiated, tissue-resident macrophages. One methodological consideration to consider in the interpretation of these data and other similar studies is the presence or absence of M-CSF in the culture media. Until recently, macrophages were believed to be terminally differentiated cells with no capacity to proliferate. We now know that at least in some experimental models, macrophage proliferation is fundamental to renewal and dependent on the presence of M-CSF or other cytokines [10]. The presence of M-CSF affects the transcriptional response to LPS, and LPS stimulation blocks M-CSF-driven proliferation. As macrophages produce M-CSF in response to inflammation, studies of transcriptional responses of mature, fully differentiated macrophage populations have demonstrated a characteristic “CSF signature” in the transcriptional responses to LPS or other inflammatory stimuli. Thus, the addition of physiological levels (10–20 ng/ml) of M-CSF may be a universal requirement in studies of LPS responsiveness, irrespective of the source of macrophage (e.g., bone marrow, alveolar, peritoneal). This could be tested by the comparison of the transcriptional profile of macrophages isolated from in vivo studies of LPS stimulation to determine whether their transcriptional profile has a distinctive CSF signature that is absent in in vitro studies that do not supplement with M-CSF.

The cytokine milieu influences LPS responses, and the IFN family of cytokines is particularly effective in this regard. The synergistic effect of IFN-γ to enhance microbicidal activity of mouse macrophages has been known for 30 years, and this occurs, in part, at the transcriptional level [11]. More recently, the discovery that LPS stimulates type I IFN (IFN-β) production through MyD88- independent signaling from the endosome is a fascinating but still poorly understood function of the LPS
response. In general, the functions of the IFN-responsive genes in antibacterial immunity are not well-characterized, although they profoundly affect the outcome of a number of bacterial infections [12]. The dissection of IFN-responsive genes that are expressed as a result of autocrine IFN-β signaling versus those that are MyD88-dependent and IFN-β-independent is a challenge, especially as the C57BL/6 mouse appears to have considerable basal production of type I IFN and a transcriptional signature to match. The authors’ investigation on the role of the type I and II IFNs in LPS signaling provided some surprises, including a subset of genes that was induced in response to IFN-β and independent of LPS, despite the fact that LPS itself induces IFN-β (e.g., Casp2, Gpsm2, Gnat2, Klh2), and those whose expression was independent of LPS but induced by IFN-β and IFN-γ (e.g., Fgrh4, Fht2, Tmem12, Cxcl1). The mechanisms behind how the type I and II IFNs modulate transcription are still unknown, as is the biological significance of the regulation of these genes; however, this is a rich avenue for future research, especially as it becomes apparent that the type I IFN response may be a contributor to a number of bacterial infections and autoimmune conditions [12]. The authors have created a rich and robust dataset that is publicly accessible (www.macrophages.com) and can be reanalyzed to ask new questions (e.g., how closely does the IFN signature, in response to LPS, match autoimmunity models? Do differences in expression of complement explain differential susceptibility of these strains to bacterial infection?), to discover the role of un- or underannotated genes in the inflammatory response and to interrogate mechanisms of regulation and coregulation by the type I and II IFNs. For example, as cytosolic sensing of LPS and subsequent caspase 11 activation have been demonstrated to require type I IFN-induced GTPases [13], is there a transcriptional signature that accompanies this signaling? Furthermore, these well-controlled and well-described experiments can be used as a platform to design complementary experiments. For example, we know that the inflammatory response to LPS differs between the sexes and with age, and therefore, companion studies could be performed to determine how many of these gene networks are influenced by sex hormones or age. Despite being studied for over two centuries, the complexities of the host response to pyrogen/endotoxin/LPS still prove a marvel.

REFERENCES


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Editorial: You give me fever: transcriptional responses to LPS

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