Guidance on use of LPS in animal studies

SUMMARY

KEY POINTS

- Dose of LPS given in mg rather than EU. All contain endotoxin levels of not less than 500,000 EU but exact amounts vary from batch to batch. Need to standardise to EU/mg/animal and not absolute amount.

- LPS serotypes. 5 types supplied by Sigma

- Purification by different methods yield different amounts of contaminating protein and RNA, which may result in different effects

- Strain differences in sensitivity. Inbred strains are generally less sensitive than outbred

- Sex differences in sensitivity. Males are generally less sensitive than females but published data not consistent

- Age differences. Senescent mice are generally more sensitive than young mice

- Readout in response to LPS and time of measurement. % of mice surviving historically used as an end-point at T=48 or 72 h. Changes in TNF-alpha can be detected as early as 2h post injection. A change in body temp does NOT correlate with changes in cytokine levels over time.

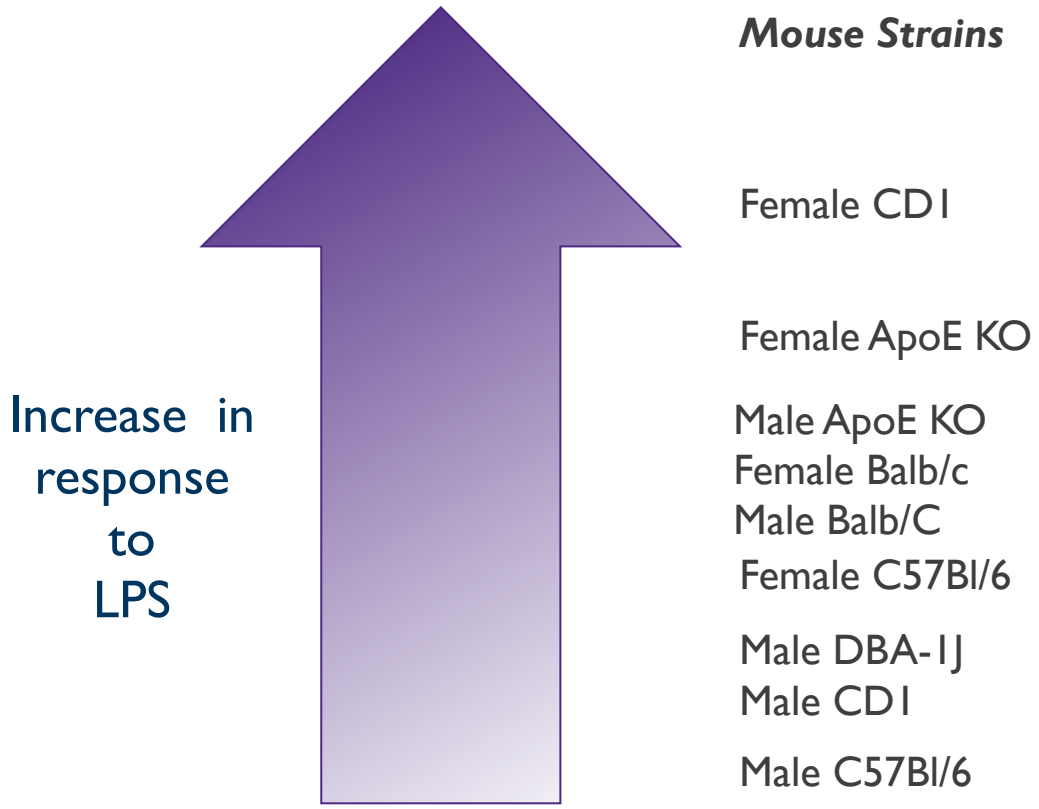

Figure 1: Schematic diagram to illustrate the susceptibility of different mouse strains and sex (all same age of 8 weeks) in response to the intra-peritoneal injection of an equivalent dose in Endotoxin Units of LPS E. coli 0111:B4 (~675,000 EU) based on a number of pro-inflammatory markers at 2 h ***(data provided by RxCelerate Limited).***

ANIMALS

Injecting mice with purified LPS via either the intraperitoneal (i.p.) route or the intravenous (i.v.) route leads to systemic activation of the innate immune system. If the dose of LPS is large enough, then the animals manifest physiological and biochemical changes that are reminiscent of certain very fulminant forms of gram-negative bacterial infection in humans, most notably overwhelming meningococcemia.

Activation of systemic inflammation by LPS in mice is largely mediated via interaction of the bacterial product with Toll-like receptor (TLR) 4, which is expressed on the surface of immune cells, such as monocytes and macrophages, as well as many other cell types, including alveolar epithelial cells and myocardial cells.

STRAIN DIFFERENCES

Strain differences in the murine system can markedly affect immunological responses. Certain inbred strains, such as C3H/HeJ mice, C57BL10/ScC, C3H/HeOuJ and TLR4-deficient mice, are hyporesponsive to LPS, whilst C3H/HeN mice are LPS-sensitive.

The LD50 for C57BL/6 mice is 25-30 mg/kg but <10 mg/kg for CD1 mice. CD-1 mice are among the most commonly used research mice as an outbred stock.

Nikodemova *et al.* Neuroscience 2011;190:67-74. Outbred ICR/CD1 mice display more severe neuroinflammation mediated by microglial TLR4/CD14 activation than inbred C57Bl/6 mice.

Thomas RC *et al*. Peptideds 2014;61:55-60 In C57BL/6 mice LPS 0.85 mg/kg injection produced no septic response, whereas 1.2 mg/kg produced a profound response within 5h. In BALB/c mice, LPS 4 mg/kg produced no response, whereas 7 mg/kg resulted in a profound response within 24h. BALB/c is an albino, immunodeficient inbred strain.

Hwang *et al* Mediators of inflammation 2012; Endotoxemia was induced in BALB/c mice (male, 6-7 weeks, 20–25 g) by intraperitoneal injection of bacterial endotoxin (10 mg/kg, Escherichia coli LPS 0111:B4), and housed in a pathogen-free environment followed 30 min later by a lethal infusion of endotoxin (LPS, 10 mg/kg, i.p.) 100% lethality by 72 h.

Takaoka Y Nature 2014 LPS (i.p. 20 mg/kg) injection in BALBc mice. 100% lethality by 48h.

Compared with humans, mice, even "normally responsive" strains, are remarkably less sensitive to the toxic or lethal effects of LPS. When the gene expression profile for human endotoxemia was compared with the gene expression profile for murine endotoxemia, the Pearson correlation analysis yielded an R^2 value of 0.01 (i.e., essentially NO correlation at all!).

The biological mechanism(s) that are responsible for this enormous difference remain to be fully elucidated, but recent evidence suggests that one or more factors, which are present in murine sera, but are absent (or present in much lower concentrations) in human sera, are capable of suppressing the production of pro-inflammatory cytokines by murine (or human) mononuclear cells following stimulation with LPS or other TLR agonists, such as peptidoglycan (major component of the cell wall of gram-positive bacteria), zymosan (a glucan present in the cell wall of yeast) or bacterial DNA. One of these factors may be the iron-binding acute phase protein, hemopexin.

Mitchell P Fink. Animal models of sespsis. Virulence 2014;5:143-145

GENDER DIFFERENCES

Consistent with our data, Mabley, JPET 2005 showed female mice to be less sensitive to LPS than males (strain information not given)

Von Drygalski Arteriosclerosis, Thrombosis, and Vascular Biology. 2013; 33: 769-776 male C57BL/6 mice are less sensitive than female. E. coli (0111:B4) male mice: LD25 [5.8 mg/kg], LD40 [8.9 mg/kg], and LD50 [10.8 mg/kg]; and female mice: LD25 [3.7 mg/kg], LD40 [5.2 mg/kg], and LD50 [6.9 mg/kg]).

Queen *et al* PLOS one 2011;11:1-136 compared responses in male and female mice. Interleukin-6 (IL-6), macrophage inflammatory protein-Iβ (MIP-1β), tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) as well as *beta-fibrinogen* (*Fgb*) and *metallothionein-I* (*Mt-1)* mRNA expression were measured at four time points (0, 2, 4 and 7 hours) after injection of *E. coli* LPS (5 mg/kg but not mention of sera type) in mice from three inbred strains. (CD1, BALBc and C57BL/6). Statistical analysis using analyses of variance (ANOVAs) showed that the levels of the all six traits changed over time, generally peaking at 2 hours after LPS injection. *Mt-1*, *Fgb* (stress gene markers), and IL-6 showed differences among strains, although these were time-specific. Sexual dimorphism was seen for *Fgb* and IL6, and was most pronounced at the latest time period (7 hours) where male levels exceeded those for females. Trends for all 6 cytokines/gene expression traits were negatively correlated with those for body temperature. The higher levels of expression of Fgb and IL6 in males compared with females are consistent with the greater vulnerability of males to infection and subsequent inflammation. TNF-alpha was highly expressed at 2 hours following LPS in all mice regardless of sex or strain (we have contradictory data to this).

Marriot I *et al.* J of Reproductive Immunology 2006;71:12-27 Gender-based differences in the incidence and severity of bacterial sepsis render males more susceptible to septic shock than females. However, the mechanisms that underlie this sexual dimorphism remain unclear. In the present study we confirm that males produce significantly higher levels of the inflammatory cytokine IL-6 and the acute phase protein LPS-binding protein (LBP) than females following in vivo lipopolysaccharide (LPS) exposure. It has also been verified that LPS-challenged male-derived macrophages produce higher levels of IL-1β and lower levels of PGE_2 than similarly treated female-derived cells. Importantly, we demonstrated that male-derived macrophages produce significantly higher levels of the inflammatory chemokine IP-10 following LPS challenge than their female counterparts. It has been demonstrated further that, although resting macrophage levels of mRNA encoding Toll-like receptor 4 (TLR4) and its co-receptor CD14, are not significantly different between genders, male-derived macrophages constitutively express higher levels of these proteins on their cell surface. Elevated circulating levels of LBP and constitutively higher cell surface expression of TLR4 and CD14 on macrophages in males could result in the observed sexual dimorphism in LPS-induced inflammatory mediator production and the greater susceptibility of males to bacterial sepsis.

AGE

Saito H *et al* Mech Ageing Dev. 2003;1241047-58. Their results clearly demonstrated an age-associated increase in mortality, hypothermia, and induction of IL-6 during endotoxemia and sepsis.

Chorinchath BB *et al* J immunol 1996:156;1525-1530 Three ages of male B6JC3J/Nia mice, young (2 mo old), mature (12 mo old), and senescent (24 mo old), were treated with bacterial LPS, and the older mice were found to be 10 times more sensitive to LPS lethality. Increased sensitivity to LPS in senescent mice correlated with significantly elevated plasma TNF-alpha and nitric oxide levels.

Another study induced endotoxemia by LPS and compared mortality, degree of hypothermia and thrombosis in three animal groups; young mice with a low dose of LPS (2.5 mg/kg resulting in no mortality in 1 week), young mice with a higher dose of LPS (20 mg/kg resulting in 78% mortality in 1 week), and aged mice with the same low dose of LPS (2.5 mg/kg resulting in 80% mortality in 1 week). The aged mice (given low LPS dose) and young mice given high LPS dose showed almost identical mortality rates and degrees of hypothermia, which were both significantly higher than in young mice with low LPS dose, suggesting that profound hypothermia under systemic inflammation is dependent on the severity of disease, rather than age.

LPS SEROTYPES

Sigma sell different serotypes of E.coli. Below is information shown currently on their website:
055:B5 (the source strain is CDC 1644-70) shown to stimulate human peritoneal macrophages at 1ng/ml
011:B4 (the source strain is from a private collection has been used to stimulate B-cells and induce NOS in human hepatocytes.
0127:B8 (the source strain is ATCC 12740. This LPS serotype has been used in the study of septic shock and to induce NOS in murine macrophages and PAF synthesis in rat glomerular mesangial cells
0128:B12 (the source strain is CDC 2440-69)
026:B6 LPS from serotype 026:B6 is the only LPS from E.coli which exhibits short chain-length behaviour on SDS-PAGE. The short chain-length of this LPS is closer to that of the mutant rough strain LPS. This LPS serotype has been used at 1 μg/ml to stimulate neutrophils.

Sigma's lipopolysaccharides contain endotoxin levels of not less than 500,000 EU (endotoxin units)/mg unless otherwise noted but the exact amount can only be found by searching for it on the certificate of analysis and amounts can and ***will*** vary from batch to batch. Most users quote giving doses in mg/kg rather than absolute units of endotoxin – this is wrong!

LPS can be prepared by TCA, phenol, or phenol-chloroform-petroleum ether (for rough strains) extraction. The TCA extracted lipopolysaccharides are structurally similar to the phenol extracted ones. Their electrophoretic pattern and endotoxicity are similar. The main differences are in the amounts of nucleic acid and protein contaminations. The TCA extract contains approximately 2% RNA and approximately 10% denatured proteins. The phenol extract contains up to 60% RNA and less than 1% protein. Purification by gel filtration chromatography removes much of protein present in the phenol-extracted LPS, but leaves a product that still contains 10-20% nucleic acids. Further purification using ion exchange chromatography, will yield an LPS product which contains <1% protein and <1% RNA.

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