REVIEW

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Lipopolysaccharide hyporesponsiveness: protective or damaging response to the brain?

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Abstract

Lipopolysaccharide (LPS) endotoxins are widely used as experimental models of systemic bacterial infection and trigger robust inflammation by potently activating toll-like receptors 4 (TLR4) expressed on innate immune cells. Their ability to trigger robust neuroinflammation despite poor brain penetration can prove useful for the understanding of how inflammation induced by viral infections contributes to the pathogenesis of neurodegenerative diseases. A single LPS challenge often result in a blunted inflammatory response to subsequent stimulation by LPS and other TLR ligands, but the extent to which endotoxin tolerance occur in the brain requires further clarification. LPS is also thought to render the brain transiently resistant to subsequent brain injuries by attenuating the concomitant pro-inflammatory response. While LPS hyporesponsiveness and preconditioning are classically seen as protective mechanisms limiting the toxic effects of sustained inflammation, recent research casts doubt as to whether they have beneficial or detrimental roles on the brain and in neurodegenerative disease. These observations suggest that spatio-temporal aspects of the immune responses to LPS and the disease status are determinant factors. Endotoxin tolerance may lead to a late pro-inflammatory response with potential harmful consequences. And while reduced TLR4 signaling reduces the risk of neurodegenerative diseases, up-regulation of anti-inflammatory cytokines associated with LPS hyporesponsiveness can have deleterious consequences to the brain by inhibiting the protective phenotype of microglia, aggravating the progression of some neurodegenerative conditions such as Alzheimer's disease. Beneficial effects of LPS preconditioning, however appear to require a stimulation of anti-inflammatory mediators rather than an attenuation of the pro-inflammatory response.

Keywords: lipopolysaccharide, neuroinflammation, endotoxin tolerance, cytokines, neurodegenerative diseases.

Introduction

Lipopolysaccharide (LPS) endotoxins, the major outer membrane components of Gram-negative intestinal microbiota, are potent activators of innate immunity, and as such widely used as experimental models of systemic bacterial infections. LPS typically consists in lipid A and polysaccharides or oligosaccharides, with the saccharides element being diverse in length and composition amongst the different Gram-negative bacteria species [1]. Lipid A molecules trigger the biosynthesis of diverse mediators of inflammation by potently activating toll-like receptors 4 (TLR4) expressed on innate immune cells, but other proteins, including TLR2, also bind LPS with minimal contribution to its effects [2]. TLR4 are part of the large mammalian TLRs family consisting of at least 11 receptors in human and 13 in mice. They are expressed on most tissues [3] predominantly on immune cells, thereby playing a crucial role in the control of the immune response to pathogens [4].

In the central nervous system (CNS), all cell types, including neurons, express at least one TLR, but microglia, the resident immune cell of the brain, expresses the whole repertoire and TLR4 selectively [5–7]. Microglial activation is a hallmark of brain pathology, which contributes to the neuroinflammation-related neuronal injury in neurodegenerative diseases when prolonged and/or uncontrolled, while also having beneficial effects through its phagocytotic phenotype [8–10]. Thus, by targeting microglia, LPS can be seen as model of choice for the understanding of the complex interplay between infection, compromised microglial function, neuroinflammation and neurodegeneration in brain diseases, but its poor brain penetration upon systemic administration has to be taken into account [11]. Systemic LPS administration nevertheless induces robust microglial activation and neuroinflammation [12]. Understanding how can provide new insights into the emerging view that inflammation resulting from viral infections is a trigger of the clinical onset of neurodegenerative diseases [13, 14].

To model chronic inflammation, repeated LPS administrations would be an appealing approach, but LPS hyporesponsiveness, also called "endotoxin tolerance" [15], is a frequent outcome also induced by genetic mutations of TLR4 [16, 17]. The concept of endotoxin tolerance was developed after the observation that pre-exposure to a sublethal dose of LPS markedly reduced mortality in animals re-challenged with a lethal dose of LPS while also inducing cross-tolerance to others TLRs ligands [18, 19]. Endotoxin tolerance is observed in the clinical settings [19] where its implications for a range of inflammatory conditions have been well described in most peripheral tissues, with both protective – enhanced resistance to sepsis and ischemia – and detrimental – increased risk of secondary infections in patients with non-infectious systemic inflammation response syndrome – consequences [19, 20]. Its impact on the brain, however, remains poorly understood. Endotoxin tolerance also manifest with transient protection to subsequent brain injuries resulting from the associated blunted neuroinflammatory response [21], but the occurrence of beneficial effects of preconditioning with sub-lethal LPS doses

may be disease-dependent. Thus, the purpose of this review is to question the impact of endotoxin tolerance on the brain following systemic LPS administration and to shed light on the detrimental or beneficial roles that LPS hyporesponsiveness and preconditionning may have in neurodegenerative diseases. To address these questions, it is important to focus on timing, therefore the time course of immune responses to LPS will first be investigated.

How do peripheral LPS trigger immune responses in the brain?

LPS dose-dependently induces inflammation to severe sepsis associated with a sickness syndrome. The immune response to LPS response typically consists in the secretion of pro-inflammatory cytokines, including interleukins (IL, *e.g.*, IL-1β, IL-6, IL-8, and IL-12), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ); anti-inflammatory cytokines [*e.g.*, IL-1 receptor antagonist (IL-1Ra), IL-4, IL-10, IL-13] secreted as feedback inhibitors to terminate the LPS response as well as acute phase proteins [*e.g.*, C-reactive protein (CRP), serum amyloid A, serum amyloid P component]. IL-1β is classically seen as a signature of inflammation since it is normally present a very low levels and very rapidly induced in response to pro-inflammatory stimuli [22].

Upon systemic LPS administration, immune mediators are primarily synthesized in the periphery. They are also found in increased levels in the brain in response to LPS, but their origin is a matter of debate. There is indeed controversy as to whether LPS crosses the blood–brain barrier (BBB) and can mediate an immune response *via* direct stimulation of microglial TLR4. Brain concentrations of LPS were indeed found to be about 0.025% of intravenously administered doses per gram of tissue at two hours post-injection [11]. This suggests that transit of circulating cytokines across the BBB after LPS challenge is the most likely potential source of cerebral cytokines levels, indirectly inducing resident microglia to produce the same cytokines through a TLR4-independent mechanism [23, 24]. The presence of TLR4 on CNS-resident cells, particularly perivascular hematopoietic cells, is however required for sustained neuroinflammation after systemic LPS administration independent of systemic cytokines [7]. Some cytokines can also be produced through direct microglial stimulation by LPS in brain structures devoid of BBB such as the circumventricular organs [25]. Moreover, LPS has been shown to increase BBB permeability *via* binding to TLR4 in endothelial cells at the surface of blood vessels, indirectly activating adjacent microglia and potentially inducing late disruption of the BBB *via* some of the secreted cytokines (*e.g.*, TNF-α, IL-1β) [26, 27]. A direct delayed stimulation of microglia by LPS can therefore be expected [7] and this process may be exacerbated in neurodegenerative diseases where the BBB is compromised [28].

The CNS mediates the sickness response to LPS, which is characterized by flu like-symptoms including reduced motivation for food and fluids, behavioral suppression, social withdrawal, hyperalgesia and changes in core body temperature (fever in human, often hypothermia in mice). These physiological and behavioral changes are considered a protective response designed to facilitate recovery from the infection [29]. Sickness behavior was long thought to be driven by the secreted pro-inflammatory cytokines, but its occurrence at LPS doses below the threshold for inducing inflammation and its advanced time course compared to that of most circulating inflammatory mediators led to the proposal that other mechanisms, such as vagal nerve stimulation or blood-borne prostaglandin E2, contribute to its onset [30, 31].

When do brain immune responses occur after systemic LPS?

The most comprehensive time courses come from studies in human healthy volunteers showing that LPS dose-dependently increases physiological indicators of sickness, and blood levels of cytokines and acute phase proteins. These experiments [32–38], summarized in Table 1, indicate that the inflammatory response to effective LPS doses (>0.3 ng/kg, i.v.) is transient, usually resolving within 24 hours, and biphasic with an early pro-inflammatory phase followed by an anti-inflammatory phase. The time course of the LPS response vary between individual symptoms, immune mediators and dose used (Table 1), but it can be concluded that (*i*) sickness symptoms initiate within one hour, peak between two and six hours and slowing resolve after 12 hours, (*ii*) circulating pro-inflammatory cytokines levels typically peak at 1.5–3.5 hours and resolve after 6–12 hours when levels of CRP levels start to gradually raise until at least 24 hours post-LPS, while (*iii*) anti-inflammatory cytokines peak from two hours post-LPS administration and remain persistently and moderately elevated levels for at least eight hours post LPS. The most rapid changes are seen with TNF-α, which peaks first and start returning to baseline levels before other pro-inflammatory cytokines reach their peak response.

Time course information in rodents are usually restricted to 2–4 time points, but tend to indicate that the peripheral (Table 2) and sickness (Table 3) responses to LPS follow a similar temporal pattern showing that (*i*) sickness behavior, with noticeable locomotor suppression during the dark-active phase of the circadian cycle and increase in body temperature during the light-inactive phase of the day, persists for over 24 hours, (*ii*) levels of pro-inflammatory cytokines are elevated between two and six hours post-injection, and (*iii*) levels of anti-inflammatory cytokines appear to be more persistently elevated.

The poor brain penetration of systemic LPS strongly argues in favor of a delayed brain immune response as compared to the periphery, but this cannot be determined with certainty as direct comparisons in rodents often include too few time points and quantify mRNA rather than protein levels of central immune mediators. Changes in mRNA levels can be induced very rapidly (Table 3), but it is unclear whether, and to which extent, they would later be translated into protein. Indeed, a recent *in vitro* study peripheral blood mononuclear cells incubated with the VLP (virus-like particle) influenza A virus showed that protein changes can only be predicted by mRNA levels for a limited number of the 20 cytokines and chemokines investigated [39]. The authors found that mRNA levels closely mirror protein levels for IFN-γ, MIP1A (macrophage

inflammatory protein-1alpha), IP10 (interferon gamma-induced protein-10), and TNF-α; moderately parallel protein levels for IL-2, GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-5, RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), and MCP1 (monocyte chemoattractant protein-1) but were unrelated to protein levels for the 11 other markers [IL-1A, IL-1B, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IL-17B, G-CSF (granulocyte-colony stimulating factor), and EOTAXIN]. The data reviewed in Tables 2 and 3, however allow to conclude that IFN-γ is only induced in the periphery, while IL-6 is the most responsive pro-inflammatory cytokine in both compartments. One study directly compared serum and whole brain cytokine levels in the same assay after a high non-lethal dose of 3 mg/kg of LPS [40], pointing towards a distinct time course in the brain rather than a delayed response. Indeed, while IL-10 showed a similar biphasic response in both compartments, characterized by an early (within four hours) and late (after 24 hours) increase, a delayed brain increase was observed for IL-1α, IL-1β and TNF-α, but the elevation in IL-6 levels was more persistent in the brain than in the blood [40].

Table 1 – ***Time course of sickness syndrome and peripheral immune mediators in healthy volunteers subjected to single and repeated LPS challenges***

	1 h	1.5 h	2 h	2.5 h	3 h	3.5 h	4 h	4.5 h	5 h	6 h	6.5 h	8 h	10 h	12 h	20 h	24 h	Dose / Reference
Sickness syndrome																	
Symptom scores	=	++	+++	+	+		+	+	=	=						=	2 ng/kg [34]
	=	++	++	=	=												2*2 ng/kg [34]
	=	=	+	=	=												3*2 ng/kg [34]
	=	=	=	=	=												4*2 ng/kg [34]
	=	=	=	=	=		=	=	=	=							5*2 ng/kg [34]
Body temperature	=	=	=		+		+++			+		=	=		=	=	0.5 ng/kg [32]
	=	=	++		+++		+++			++		+	+		=	=	1 ng/kg [32]
	=	+	++	++	+++	+++	+++	+++	++	++		+	+		=	=	2 ng/kg [32, 34, 41]
	=	+	++														2*2 ng/kg [34]
	=	=	+														3*2 ng/kg [34]
	=	=	+														4*2 ng/kg [34]
	=	=	+		+		++	++	+	+							5*2 ng/kg [34]
	+	+	++	++	+++	+++	+++	+++	+++	++	++	++				+	4 ng/kg [33, 35]
Heart rate	=	=	=		+		+++			+		+	=		=	=	0.5 ng/kg [32]
	=	=	+		+++		++			++		++	+		=	=	1 ng/kg [32]
	+	+	+		++		++	+++	+++	+++		++	+		=	=	2 ng/kg [32, 34]
	=		=		+		++	++	+	+							5*2 ng/kg [34]
	=	+	++	++	+++	+++	+++	+++	+++	++	++	++		++		+	4 ng/kg [33, 35]
Pro-inflammatory cytokines																	
TNF-α			+++				++			+		+	=				0.4 ng/kg [38]
	=	=	=		=		=			=							0.5 ng/kg [32]
	+	+++	+++		+		+			=							1 ng/kg [32]
	+	+++	+++		++		+		+	=		=					2 ng/kg [32, 34, 36, 37, 41]
	=	=	=				=			=							5*2 ng/kg [34]
	+	++	+++	+++		++		++			+	+				=	4 ng/kg [33]
IFN-γ	+	+++	++		=		=										2 ng/kg [36]
IL-6			+++				++			=		=	=				0.4 ng/kg [38]
	=	=	=		+++		=			=							0.5 ng/kg [32]
	=	=	+++		+++		=			=							1 ng/kg [32]
	=	+	+++		+++		+		=	=		=					2 ng/kg [32, 34, 36, 37, 41]
	=	=	=				=			=							5*2 ng/kg [34]
	=	+	++	+++		+++		+++			+	+				=	4 ng/kg [33]
IL-8	=	=	=		+++		=			=							0.5 ng/kg [32]
	=	=	+++		+++		+			=							1 ng/kg [32]
	=	+	+++		+++		++			=							2 ng/kg [32]
	=	+	++	+++		+++		++			+	+				=	4 ng/kg [33]
Anti-inflammatory cytokines																	
IL-1Ra	=	=	=				+++			+		+					2 ng/kg [34, 37]
	=	=	=				=			=							5*2 ng/kg [34]
IL-4	=	=	++		+++		+++		++	+							2 ng/kg [41]
IL-10	=	+	+++		+++		+			+		+					2 ng/kg [34, 36, 37]
	=	=	=				=			=							5*2 ng/kg [34]
TGF-β	+	++	+++				++			++							2 ng/kg [34]
	+	++	++				++			++							5*2 ng/kg [34]

	1 h	1.5 h	2 h	2.5 h	3 h	3.5 h	4 h	4.5 h	5 h	6 h	6.5 h	8 h	10 h	12 h	20 h	24 h	Dose / Reference
Acute phase proteins																	
CRP							=							+		++	0.5 ng/kg [32]
							=							++		+++	1 ng/kg [32]
	=						=			=				++		+++	2 ng/kg [32, 34, 36]
																++++	2*2 ng/kg [34]
																+++	3*2 ng/kg [34]
																++	4*2 ng/kg [34]
	=	=		=		=		=				+				+++	4 ng/kg [33]

+++ denotes peak response regardless of the dose. Significant increase from control levels: +, up to 50% of the peak response; ++, above 50% of the peak response; =, non-significant change from pre-LPS levels or untreated controls. Estimated increased or decreased magnitude of the LPS response to a 2nd challenge is indicated in red: =, lack of LPS response; ++++, increased levels after a 2nd challenge; + or ++, magnitude of the LPS response compared to a single challenge with the same dose. References: [32] Dillingh *et al.* (2014); [33] Kümpers *et al.* (2009); [34] Draisma *et al.* (2009), up to 5*2 ng/kg at 24-hour intervals; [35] Lynn *et al.* (2003); [36] Kemna *et al.* (2005); [37] van den Boogaard *et al.* (2010); [38] Straub *et al.* (2002); [41] Clodi *et al.* (2008). IL: Interleukin; TNF-*α*: Tumor necrosis factor alpha; TGF-*β*: Transforming growth factor beta; IFN-*γ*: Interferon gamma; CRP: C-reactive protein.

Table 2 – ***Time course of peripheral immune response to single and repeated systemic LPS challenges in healthy mice and rats***

	1 h	2 h	3 h	4 h	6 h	8 h	12 h	16 h	20 h	24 h	28 h	Dose / Reference
Pro-inflammatory cytokines												
TNF-*α*	++	+++		=	=							0.1 mg/kg [42, 43]
		+++						=				0.25 mg/kg [44]
		+++			+					=		0.63 mg/kg [45]
		+++			=							1 mg/kg [7]
		+++			+					=		2.5 mg/kg [45]
				+++		+		+			=	3 mg/kg [40]
				+++								3*3 mg/kg [40]
IFN-*γ*		=			+++					=		0.63 mg/kg [45]
			+++									0.5 mg/kg [46]
			=									2*0.5 mg/kg [46]
			=									3*0.5 mg/kg [46]
		=			+++							1 mg/kg [7]
		=			+++					=		2.5 mg/kg [45]
IL-1*α*				+++		+++		++			=	3 mg/kg [40]
				+++								3*3 mg/kg [40]
IL-1*β*		+++										0.1 mg/kg [43]
		+++						++				0.25 mg/kg [44]
			+++									0.5 mg/kg [46]
			+									2*0.5 mg/kg [46]
			+									3*0.5 mg/kg [46]
		+++			=					=		0.63 mg/kg [45]
		+++			+++							1 mg/kg [7]
		+++			=					=		2.5 mg/kg [45]
				++		+		=			+	3 mg/kg [40]
				++								3*3 mg/kg [40]
IL-6	=	+++		=	=							0.1 mg/kg [42, 43]
		+++						=				0.25 mg/kg [44]
		+++			++					+		0.63 mg/kg [45]
		+++			++	++				+		2.5 mg/kg [45, 47]
				+++		=		=			=	3 mg/kg [40]
				++++								3*3 mg/kg [40]
IL-12			+++									0.5 mg/kg [46]
			=									2*0.5 mg/kg [46]
			=									3*0.5 mg/kg [46]
		++			+++							1 mg/kg [7]
Anti-inflammatory cytokines												
IL-1Ra		+++										0.1 mg/kg [43]
IL-10		+++			+++					=		0.63 mg/kg [45]
		+++			+++	+++				+		2.5 mg/kg [45, 47]
				+		=		+			+++	3 mg/kg [40]
				++								3*3 mg/kg [40]

	1 h	2 h	3 h	4 h	6 h	8 h	12 h	16 h	20 h	24 h	28 h	Dose / Reference
IL-13				---		---		---			---	3 mg/kg [40]
				---								3*3 mg/kg [40]

+++ denotes peak response regardless of the dose or significantly elevated levels for single time points. Significant increase from control levels: +, up to 50% of the peak response; ++, above 50% of the peak response; =, non-significant change from control levels. Estimated increased or decreased magnitude of the LPS response to a 2nd challenge is indicated in red: =, lack of LPS response; ++++, increased levels after repeated non-lethal doses of LPS; +, ++, +++, magnitude of the LPS response compared to a single challenge with the same dose. References: [7] Chakravarty & Herkenham (2005); [40] Erickson & Banks (2011), 3*3 mg/kg within 24 hours and given at 0, 6 and 24 hours; [42] Teeling *et al.* (2010); [43] Skelly *et al.* (2013); [44] Chen *et al.* (2005); [45] Biesmans *et al.* (2013); [46] Püntener *et al.* (2012), 3*0.5 mg/kg at 24-hour intervals; [47] Schneiders *et al.* (2015). IL: Interleukin, TNF-*α*: Tumor necrosis factor alpha; IFN-*γ*: Interferon gamma.

Table 3 – ***Time course of the central immune response to single and repeated LPS challenges in healthy mice and rats***

		1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	16 h	20 h	24 h	28 h	Dose / Reference
Sickness syndrome														
Locomotion		=	=	=	=	=	=	=	=	=	=	=		50 µg/kg [47]
		=	=	=	=	=	=	--	--	--	--	--		2.5 mg/kg [47]
Body temperature		+	++	++	++	++	++	=	-	-	=	=		50 µg/kg [47]
				++										0.1 mg/kg [43]
		+	++	++	++	++	++	+	--	=	=	++		2.5 mg/kg [47]
Pro-inflammatory cytokines														
TNF-*α*	mRNA			+++										0.1 mg/kg [43]
	mRNA			=						+++				0.25 mg/kg [44]
	mRNA			+++			++					=		0.63 mg/kg [45]
	mRNA			+++		+++								1 mg/kg [7, 48]
	mRNA					+++								2*1 mg/kg [48]
	protein											+++		2 mg/kg [49]
	mRNA							+++				=		2.5 mg/kg [47]
	protein			+++			+++					=		2.5 mg/kg [45]
	protein					=		=		=			+	3 mg/kg [40]
	protein					+++								3*3 mg/kg [40]
IFN-*γ*	protein				=									0.5 mg/kg [46]
	protein				=									2*0.5 mg/kg [46]
	protein				=									3*0.5 mg/kg [46]
	mRNA			=			=					=		0.63 mg/kg [45]
	mRNA					=								1 mg/kg [48]
	mRNA					=								2*1 mg/kg [48]
	mRNA			=			=					=		2.5 mg/kg [45]
IL-1*α*	protein					=		=		+++			=	3 mg/kg [40]
	protein					+++								3*3 mg/kg [40]
IL-1*β*	mRNA	++		+++		+	+							0.1 mg/kg [42, 43]
	mRNA			+++						=				0.25 mg/kg [44]
	protein				=									0.5 mg/kg [46]
	protein				+++									2*0.5 mg/kg [46]
	protein				++									3*0.5 mg/kg [46]
	mRNA			=			=					=		0.63 mg/kg [45]
	mRNA			+++		+++	+++							1 mg/kg [7, 48]
	mRNA					++								2*1 mg/kg [48]
	protein												+++	2 mg/kg [49]
	protein			=			+++					=		2.5 mg/kg [45]
	protein					=		=		=			+++	3 mg/kg [40]
	protein					=								3*3 mg/kg [40]
Pro-IL-1*β*	mRNA							+++				+		2.5 mg/kg [47]
IL-6	mRNA	=		+++		=	=							0.1 mg/kg [42, 43]
	mRNA			+++						=				0.25 mg/kg [44]
	mRNA			+++			++					=		0.63 mg/kg [45]
	mRNA					+++								1 mg/kg [48]
	mRNA					+								2*1 mg/kg [48]
	protein												+++	2 mg/kg [49]
	mRNA							+++				=		2.5 mg/kg [47]
	protein			+++			+++					=		2.5 mg/kg [45]
	protein					+		+		+			+	3 mg/kg [40]
	protein					+++								3*3 mg/kg [40]

			1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	16 h	20 h	24 h	28 h	Dose / Reference
IL-12		protein				=									0.5 mg/kg [46]
	protein					+++									2*0.5 mg/kg [46]
	protein					=									3*0.5 mg/kg [46]
Anti-inflammatory cytokines															
IL-1Ra		mRNA					+++								1 mg/kg [48]
	mRNA						++								2*1 mg/kg [48]
	mRNA								+++				+		2.5 mg/kg [47]
IL-10		mRNA			=			=					=		0.63 mg/kg [45]
	mRNA						+++								1 mg/kg [48]
	mRNA						+								2*1 mg/kg [48]
	mRNA				=			=					=		2.5 mg/kg [47]
	protein								+++				=		2.5 mg/kg [45]
	protein						+		=		=			+	3 mg/kg [40]
	protein						=								3*3 mg/kg [40]
IL-13		protein					=		=		=			=	3 mg/kg [40]
	protein						=								3*3 mg/kg [40]

+++ denotes peak response regardless of the dose or significantly elevated levels for single time points. Significant changes from control levels: + or -, increase or decrease of up to 50% of the peak response; ++ or --, increase or decrease larger than 50% of the peak response; =, non-significant change from control levels. Estimated increased or decreased magnitude of the LPS response to a 2nd challenge is indicated in red: =, lack of LPS response; ++++, increased levels after a repeated non-lethal doses of LPS; +, ++, +++, magnitude of the LPS response compared to a single challenge with the same dose. References: [7] Chakravarty & Herkenham (2005), whole brain; [40] Erickson & Banks (2011), whole brain, 3*3 mg/kg within 24 hours and given at 0, 6 and 24 hours; [42] Teeling *et al.* (2010), dorsal hippocampus; [43] Skelly *et al.* (2013), hypothlamus and hippocampus; [44] Chen *et al.* (2005), whole brain; [45] Biesmans *et al.* (2013), whole brain; [46] Püntener *et al.* (2012), whole brain, 3*0.5 mg/kg at 24-hour intervals; [47] Schneiders *et al.* (2015), hypothalamus; [48] del Rey *et al.* (2009), hypothalamus, 2*1 mg/kg at three weeks interval; [49] Cazareth *et al.* (2014), hypothalamus and hippocampus. IL: Interleukin, TNF-α: Tumor necrosis factor alpha; IFN-γ: Interferon gamma.

In regard to the temporal response of glia, microglia are activated first and then recruit astrocytes to further propagate inflammatory signals and inhibit microglial activities [50]. The exact timing for these processes has not been studied in details. They are, however, thought to be largely mediated by IL-1β [50], which is induced in the brain during the early phase of the immune response (Table 3). These neuroinflammatory processes are also normally transient, with microglia returning to a resting state, as the immune stimulus is resolved.

LPS hyporesponsiveness or delayed pro-inflammatory response?

Recent findings cast doubt as to whether hyporesponsiveness occurs following repeated LPS challenges. Hyper-responsiveness develops under some circumstances, but more generally, the second LPS response rather seems distinct from the first occurrence, differing spatio-temporally and by the profile of secreted immune mediators.

In human, endotoxin tolerance is typically studied *ex vivo* using blood cells from healthy volunteers pre-exposed to LPS and subsequently incubated with LPS. Resulting data have to be taken with caution since non-stimulated blood cells proliferate and produce cytokines [51] and tolerance to LPS was found to resolve faster *ex vivo* than *in vivo* [52]. But, interestingly, the cytokine response elicited by *ex vivo* re-exposure to LPS was found characterized by a reduction in the levels of pro-inflammatory cytokines (*e.g.*, IL-1β, IL-6, and TNF-α) at six hours, which was followed by a sustained increased in the levels of these cytokines from 12 hours onwards, although IL-8 showed the opposite behavior [32]. Thus, this suggests that hyporesponsiveness to LPS occur in the early phase of the second challenge for some cytokines, when a reduction of the sickness syndrome associated with a attenuated increase in circulating pro- and anti-inflammatory cytokines levels is seen *in vivo* ([34], Table 1), but would be followed by a delayed, sustained pro-inflammatory reaction.

In rodents, circulating levels of pro-inflammatory cytokines were found attenuated with repeated exposure to moderate LPS doses (0.5 mg/kg, [46]) but stable or increased with high non-lethal doses (3 mg/kg [40]). The latter could either be due to the high dose or differences in the dosing regimen but the protective effect of LPS against subsequent lethal doses (*e.g.*, 32 mg/kg [44] suggests that the short inter-injection interval was responsible for the lack of tolerance. The second challenge was indeed applied six hours after the first when levels of pro-inflammatory cytokines are still significantly elevated (Table 2), possibly exacerbating the pre-existing inflammatory response. In contrast, Püntener *et al.* (2012) [46] have dosed at 24 hours intervals, during the anti-inflammatory phase of the LPS responses (Table 2), consistent with the view that anti-inflammatory cytokines, IL-10 in particular, are the principal mediators of endotoxin tolerance [21, 53].

Does LPS hyporesponsiveness occur in the brain?

Data summarized in Table 3 indicate that the brain is less likely to become tolerant to systemic infection than the periphery (Table 3). While the more severe dosing regimen (3×3 mg/kg within 24 hours) overall resulted in an exacerbated pro-inflammatory response, as was the case in the periphery (Table 2), LPS tolerance was cytokine-dependent at milder doses and associated with reduced levels of anti-inflammatory cytokines (Table 3). Importantly, elevations in IL-1β and IL-6 were only seen three hours after the second 0.5 mg/kg LPS doses leading to the hypothesis that immune cells in the brain may be primed

instead [46]. This is in apparent contradiction with the recent report of significantly reduced production of multiple pro-inflammatory cytokines and chemokines, including IL-1β, TNF-α and IL-6, by microglia of mice receiving a second 0.25 mg/kg dose LPS injection after four weeks [48], but the inter-injection interval may have again contributed to this discrepancy.

Priming, which is typically observed in neurodegenerative diseases, refers to situations where microglia proliferates and adopt an activated state, thereby becoming more susceptible to a secondary inflammatory stimulus, which will then exacerbate disease progression [54, 55]. Should repeated LPS doses prime the brain rather than inducing endotoxin tolerance, repeated systemic infections would thus has deleterious effects in susceptible individuals, predisposing to or exacerbating neurodegenerative conditions, despite peripheral tolerance [14, 46]. In favor of this hypothesis, repeated pro-inflammatory doses of LPS were found to aggravate pathological hallmarks of Alzheimer's disease in transgenic mouse models. In the 3xTg-AD mouse model, 0.5 mg/kg of LPS twice a week for six weeks exacerbated *tau* pathology while also inducing persistent microglial activation and elevation of IL-1β but amyloid plaque load, IL-6 and TNF-α levels were unaltered [56]. Amyloid pathology is however also sensitive to LPS. Increased production of amyloid-*beta* and memory impairments were induced by repeated 0.250 mg/kg LPS doses in an APP/PS1 model, but although these effects were prevented by pretreatment with an anti-inflammatory agent [57], the possibility that exacerbation of amyloid plaque load would result from LPS hyporesponsiveness also deserves some consideration, as will be discussed below.

Is LPS hyporesponsiveness protective to the brain?

Evidence supporting a protective effect of LPS hyporesponsiveness in Alzheimer's disease comes from genetic association studies. The Asp299Gly polymorphism of the TLR4 gene, associated with an attenuated receptor signaling and a blunted inflammatory response, was found to reduce the risk of late onset Alzheimer's disease by 2.7-fold [58] while a combination of polymorphisms in CD14 and LXRβ CD14 receptors known to lower the inflammatory responses of microglia to bacterial infection or LPS stimulation, reduces the risk of developing the disease by 6-fold [59]. Furthermore, inflammatory cytokines levels correlate with amyloid load in the brain of transgenic mouse models of Alzheimer's disease [60] suggesting that endotoxin tolerance can be protective to Alzheimer's disease. However, although inflammatory processes are harmful to the disease, immune activation can also be beneficial by favoring microglial phagocytosis and clearance or amyloid-*beta* [10], leading to the contradictory hypothesis that LPS hyporesponsiveness would be detrimental during the course of Alzheimer's disease. IL-10, the principal mediator of endotoxin tolerance thought to protect the brain from inflammatory damage induced by LPS by inhibiting the production of all inflammatory cytokines and down-regulating TLR4 expression [21] was recently identified as an aggravating cytokine for Alzheimer's disease, promoting amyloid deposition, synaptic dysfunction and cognitive impairments [61, 62]. Moreover, the pro-inflammatory cytokine IL-1β was found able to trigger microglial activation and reduce amyloid plaque pathology [63], while attenuating astrocyte activation accelerated plaque pathogenesis in APP/PS1 mice [64]. Thus, whether LPS hyporesponsiveness is beneficial or detrimental to Alzheimer's disease appear dependent upon the disease status. It may limit the triggering impact of viral infections on disease onset in the healthy population and exacerbate disease progression once established by inhibiting the neuroprotective phenotype of microglia associated with neuroinflammation.

Data from IL-6 deficient mice also suggest that LPS hyporesponsiveness is potentially detrimental to the brain. Direct inhibition of microglial IL-6 production facilitates recovery from LPS-induced sickness syndrome without altering circulating cytokine levels (IL-1β, IL-6, IL-10, TNF-α) [65]. IL-6 deficient mice, however, were found less responsive to LPS in the brain and blood during the acute phase of the immune response, with reduced secretion of pro-inflammatory cytokines and protection against LPS-induced spatial working memory deficit [47, 66]. They were, however, more prone to inflammation in the brain at 24 hours despite elevated IL-10 levels in both compartments and attenuated peripheral pro-inflammatory response [47]. This suggests that LPS hyporesponsiveness can be associated with a delayed pro-inflammatory response to the brain which, and if sustained, harmful effects may occur [12].

The spontaneously LPS-hyporesponsive C3H/HeJ mouse: protected or susceptible to neurodegenerative conditions?

Inbred C3H/HeJ mice bear a loss of function in the TLR4 gene [67] making them refractory to LPS in the periphery because their macrophages do not produce pro-inflammatory cytokines in response to LPS [68, 69], and in the brain because of a defect in microglia to induce pro-inflammatory cytokines [70, 71]. Intracerebral LPS administration fail to induce pro-inflammatory cytokines in the brain and blood of C3H/HeJ mice [72], or behavioral sickness symptoms, which can nevertheless be triggered by an intracerebral IL-1β challenge [73]. Furthermore, the resistance of C3H/HeJ mice to LPS is associated with increased susceptibility to bacterial infections [74] and may thus be restricted to TLR4 ligands.

Consistent with their LPS-resistant phenotype, C3H/HeJ mice were found protected from conditions associated with low-grade inflammation, such as obesity and insulin-resistance induced by high fat diet [69, 75], which are known to be predominantly mediated by TLR4 [76, 77]. Interestingly, these metabolic conditions are established risks factors for Alzheimer's disease [78], suggesting that C3H/HeJ mice may be protected from age-related Alzheimer's-like changes. Under non-stimulated conditions, hippocampal neurogenesis also differs in adult C3H/HeJ mice as they produce more neurons and less astrocytes than control mice [79]. Therefore, one may expect resistance to neuroinflammation in these mice, as well as preserved cognitive ageing, but the fact that they

carry the retinal neurodegeneration mutation makes the latter difficult to assess [80]. There is, however, evidence to suggest that the C3H/HeJ mouse is not protected from neuroinflammation when mediated by stimuli other than TLR4 agonists. For instance, up-regulation of pro-inflammatory transcripts, including IL-1β, were found primarily around the blood vessels of both WT and C3H/HeJ mice exposed to commercially available LPS, but C3H/HeJ mice were refractory to inflammation induced by purified LPS, suggesting that they can mount a pro-inflammatory response in a TLR4 independent manner [7]. Non-LPS immune stimuli indeed activate microglia in the spinal cord [81] and hypothalamus of these mice while also inducing pro-inflammatory cytokines levels [82]. And although C3H/HeJ mice are protected from brain damage and neuroinflammation induced by experimental stroke [83], they were found more susceptible to prion diseases [84] and spinal cord injury [85], both of which also have an inflammatory component.

LPS preconditioning: protection or susceptibility to secondary injuries and neurodegenerative diseases?

The benefits of LPS preconditioning have been particularly well documented for stroke, in which the acute inflammation triggered by cerebral ischemia exacerbates primary brain damage. Ischemic tolerance in the brain is contributed for by inhibition of TLR4 and pro-inflammatory cytokines, and bears striking similarities with endotoxin tolerance [21]. A single pre-treatment with a very low 50 μg/kg dose of LPS 24 hours before inducing focal cerebral ischemia limited the extent of vascular injury and infarct volume too [86]. Persistent protective effects on infarct volume associated with reduced microglial activation and secretion of pro-inflammatory cytokines are commonly found at higher pro-inflammatory doses up to 200 μg/kg [87]. Higher acute LPS doses (0.2–1 mg/kg) administered 72 hours prior to middle cerebral artery occlusion, also had protective effects involving redirection of TLR signaling with an increased anti-inflammatory phenotype which was, paradoxically, not associated with a suppression of the pro-inflammatory response [88, 89]. A significantly more severe pre-treatment regimen (four daily 1 mg/kg LPS doses) also attenuated lesion volume and apoptosis after cryogenic brain injury while concomitantly increasing proliferation of microglia [23], which may have thus become primed. This suggests that the beneficial effects of LPS preconditioning are predominantly mediated by a stimulation of anti-inflammatory mediators.

In contrast, LPS can be neurotoxic in brain areas innervated by the dopaminergic system, particularly the *substantia nigra*, where microglial activation and its neurotoxic products, including pro-inflammatory cytokines, are thought to play an essential role in dopaminergic neurodegeneration during the pathogenesis of Parkinson's disease [90]. Intra-nigral administration of LPS leading to localized microgliosis was accompanied by nigrostriatal neurodegeneration and stable spontaneous motor deficits [91]. Furthermore, intra-nigral injection of a low dose of LPS, which increased pro-inflammatory cytokines levels and reduced anti-inflammatory cytokines levels in the substantia nigra, exacerbated the magnitude of cell loss produced by subsequent intra-striatal injection of the neurotoxin 6-hydroxydopamine (6-OHDA) [92], indicating that these areas of the brains are resistant to the anti-inflammatory effects of LPS preconditioning.

Conclusions

Neuroinflammation resulting from systemic viral infection can play an important role in the development of neurodegenerative diseases. This can be modeled using LPS endotoxin through stimulation of TLR4 expressed on immune cells, triggering robust neuroinflammation, despite poor brain penetration. Tolerance to LPS and other TLR ligands, mediated by an up-regulation of anti-inflammatory cytokines, typically develop upon repeated LPS stimulation, and is considered as a protective response. The observations presented above, however, point towards a distinct response, involving a delayed pro-inflammatory reaction, rather than a blunted inflammatory response. Endotoxin tolerance is also less likely to occur in the brain than in the periphery conferring susceptibility to neurodegenerative diseases, the risk of which is reduced by attenuated TLR4 signaling. LPS hyporesponsiveness can, however, have deleterious effects in the brain, whereby anti-inflammatory cytokines inhibit the protective phenotype of microglia, resulting in exacerbation of some neurodegenerative conditions such as Alzheimer's disease. Beneficial effects of LPS preconditioning, on the contrary, appear to require a stimulation of anti-inflammatory mediators rather than an attenuation of the pro-inflammatory response.

Conflict of interests

The author declares that there is no conflict of interests.

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