



Cite this: *Integr. Biol.*, 2015, 7, 1339

## On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death†

Douglas B. Kell\*<sup>a</sup> and Ethersia Pretorius\*<sup>b</sup>

We have recently highlighted (and added to) the considerable evidence that blood can contain dormant bacteria. By definition, such bacteria may be resuscitated (and thus proliferate). This may occur under conditions that lead to or exacerbate chronic, inflammatory diseases that are normally considered to lack a microbial component. Bacterial cell wall components, such as the endotoxin lipopolysaccharide (LPS) of Gram-negative strains, are well known as potent inflammatory agents, but should normally be cleared. Thus, their continuing production and replenishment from dormant bacterial reservoirs provides an easy explanation for the continuing, low-grade inflammation (and inflammatory cytokine production) that is characteristic of many such diseases. Although experimental conditions and determinants have varied considerably between investigators, we summarise the evidence that in a great many circumstances LPS can play a central role in all of these processes, including in particular cell death processes that permit translocation between the gut, blood and other tissues. Such localised cell death processes might also contribute strongly to the specific diseases of interest. The bacterial requirement for free iron explains the strong co-existence in these diseases of iron dysregulation, LPS production, and inflammation. Overall this analysis provides an integrative picture, with significant predictive power, that is able to link these processes *via* the centrality of a dormant blood microbiome that can resuscitate and shed cell wall components.

Received 8th June 2015,  
Accepted 27th August 2015

DOI: 10.1039/c5ib00158g

[www.rsc.org/ibiology](http://www.rsc.org/ibiology)

### Insight, innovation, integration

The Biological Insight of this manuscript is that while dormant bacteria, including those in blood, are normally unnoticed (as they are invisible to conventional methods of culture), they can by definition be resuscitated and then proliferate for at least a certain number of generations before possibly returning to a state of dormancy. This allows a continuing production and shedding of potent inflammatory agents such as the lipopolysaccharide (LPS) characteristic of the Gram-negative cell wall. Well-established pathways link LPS (*sensu lato*) to inflammatory cytokine production, and to cell death *via* apoptosis, programmed necrosis, and pyroptosis, with the accompanying microparticle formation known to occur with these cell death mechanisms. Cytokine-mediated cell death mechanisms that permit both (i) the translocation of bacteria between blood and other tissues, and (ii) localised proliferation leading to inflammation and cell death, are likely to be a major component of the various disease manifestations involved. One established requirement for bacterial resuscitation and proliferation comes from the need for available iron. The Technological Innovation is the use of advanced microscopy techniques to detect these dormant bacteria as well as microparticle formation. The Benefit of Integration comes (i) from bringing together these multiple biochemical elements (bacterial dormancy and resuscitation, LPS-induced inflammatory cytokine production, cytokine-induced cell death, cell-death-induced translocation, and localised cell death induced by LPS), and (ii) by showing their commonality, and the centrality of LPS, across a range of chronic, inflammatory diseases normally considered to lack a microbial component.

<sup>a</sup> School of Chemistry and The Manchester Institute of Biotechnology, The University of Manchester, 131, Princess St, Manchester M1 7DN, Lancs, UK. E-mail: [dbk@manchester.ac.uk](mailto:dbk@manchester.ac.uk); Web: <http://dbkgroup.org/>; Tel: +44 (0)161 306 4492

<sup>b</sup> Department of Physiology, Faculty of Health Sciences, University of Pretoria, Arcadia 0007, South Africa. E-mail: [resia.pretorius@up.ac.za](mailto:resia.pretorius@up.ac.za); Tel: +27 12 420 2864

† Paper 3 in the series “The dormant blood microbiome in chronic, inflammatory disease”.

## Introduction

Blood is normally considered a sterile environment in the sense of lacking active microbes, since any bacteraemia or sepsis is potentially extremely life-threatening.<sup>1</sup> However, this does not exclude the presence in blood of dormant bacteria, that by definition<sup>2,3</sup> are not growing but resist detection by standard culture techniques, yet are not ‘dead’ as they may be resuscitated



and then proliferate. We have recently summarised the considerable evidence<sup>4,5</sup> to the effect that human blood contains an authentic but dormant microbiome that can contribute significantly to a large variety of chronic inflammatory diseases, a set of diseases that is strikingly similar to those for which we had previously noted the presence of iron dysregulation<sup>6–10</sup> and hypercoagulability.<sup>9</sup>

Given the well-established facts (i) that microbial growth *in vivo* is normally strongly limited by the (non-) availability of free iron (*e.g.* ref. 11–21), and (ii) that bacterial components such as lipopolysaccharide (LPS) are strongly inflammatory (*e.g.* ref. 22 and 23), such an analysis leads to the recognition that the iron-related inflammatory diseases also have a major microbial component involving the resuscitation of dormant organisms and their shedding of inflammatory molecules, and especially of cell wall components such as LPS (Fig. 1). LPS is commonly known as endotoxin, albeit that it is frequently shed, and we shall use this name interchangeably unless otherwise specified. Most work has been done with LPS from Gram-negative bacteria, but unless specified, we recognise that much of what we have to say should be taken to apply to inflammatory processes catalysed by cell wall components (such as lipoteichoic acids<sup>24</sup>) from Gram-positive organisms, ultramicrobacteria,<sup>25</sup> and potentially (though there seems to be relatively little work on this<sup>26–29</sup>) from the cell envelopes of archaea. Also, though many of the ideas developed here very likely apply to them too, and there is a considerable literature, we shall not discuss viruses,<sup>30</sup> nor mycoplasmas<sup>31–33</sup> in much detail.

The earlier overviews<sup>4,5</sup> recognised that the chief sources of the blood microbiome were likely to be *via* translocations of microbes from the gut and oral cavities, and although a number of the diseases discussed were neurodegenerative in nature, we did not look at the evidence (and mechanism) for the transport of cells from blood into tissues such as the CNS. A chief purpose of the present review is thus to take a systems

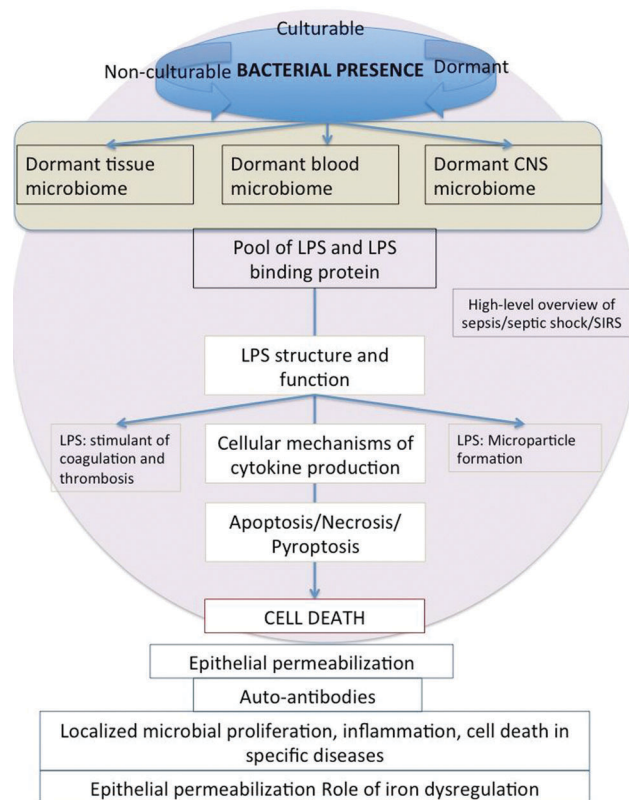


Fig. 1 An overall illustration of the headline processes involved in chronic inflammation and disease aetiology mediated *via* the resuscitation of dormant microbes and the increased production and shedding of cell wall components.

approach, designed to bring together the evidence for the strong involvement of microbes and their inflammatory bacterial cell wall components in both the diseases themselves and their dynamics, and relating the known ability of LPS and related



**Douglas B. Kell**

*Douglas Kell is Research Professor in Bioanalytical Science at the University of Manchester, UK. His interests lie in systems biology, iron metabolism and dysregulation, microbial dormancy, cellular drug transporters, synthetic biology, e-science, chemometrics and cheminformatics. He was Director of the Manchester Centre for Integrative Systems Biology prior to a 5-year secondment (2008–2013) as Chief Executive of the UK Biotechnology and Biological Sciences*

*Research Council. He is a Fellow of the Learned Society of Wales and of the American Association for the Advancement of Science, and was awarded a CBE for services to Science and Research in the New Year 2014 Honours list.*



**Etheresia Pretorius**

*Resia Pretorius is a Research Professor in the Department of Physiology, Faculty of Health Sciences at the University of Pretoria, South Africa. Her interests lie in the ultrastructure and regulation of the human coagulation system, with particular focus on erythrocytes and fibrin networks, the role of iron metabolism and changes to the coagulation system due to inflammation. She is also Director of the Applied Morphology Research*

*Centre of the University of Pretoria. She was chosen as the winner in 2011 of the African Union Kwame Nkrumah Scientific Awards Program: Women Scientist Regional Awards in the category Basic Science and Technology.*



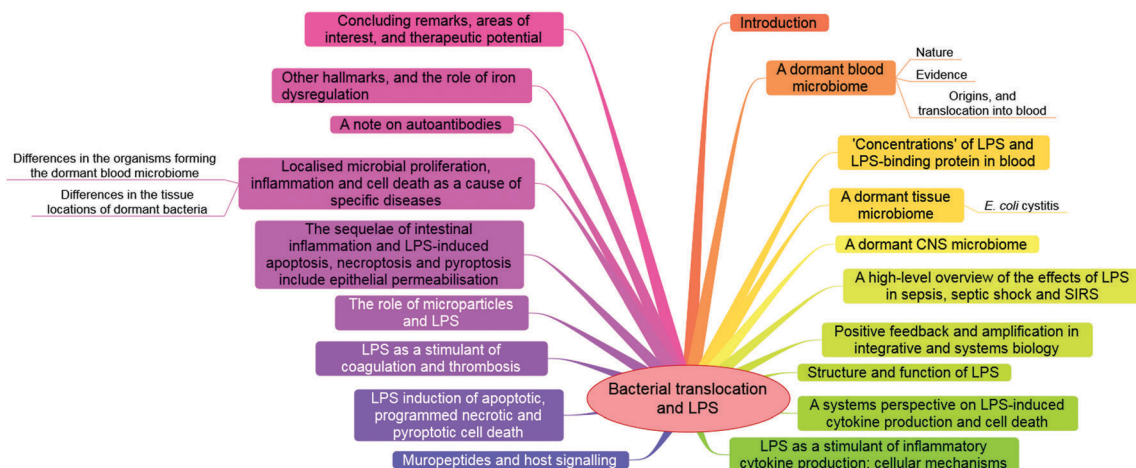


Fig. 2 A 'mind map'<sup>34</sup> summarising the article.

component to induce (mainly apoptotic) cell death. This turns out to be sufficient to explain many of the acute and chronic sequelae of the presence of microbes and their cell envelope products in mammals. An overview of the present article is given in the form of a mind map (Fig. 2). As background, we first discuss microbial dormancy, culturability and non-culturability.

## A dormant blood microbiome

### Nature

As foreshadowed in the introduction, we<sup>4,5</sup> and others<sup>35,36</sup> have summarised the rather extensive evidence that the presence of bacteria in blood is – perhaps unsurprisingly, given the assumption that blood is normally sterile – much commoner than is usually supposed, and we have pointed out<sup>4,5</sup> that, in the usual absence of overt bacteraemia, such organisms are best considered as being in a dormant physiological state.

Dormancy is defined as a reversible non-replicating state, often of low metabolic activity. Leaving aside obviously specialised forms such as spores and seeds ('constitutive dormancy'<sup>2</sup>), in non-sporulating bacteria it manifests typically as an inability of an individual cell to proliferate (*e.g.* to form a colony) under conditions normally considered adequate for cultivation, but where the cell is not operationally 'dead'<sup>3</sup> in that it can revert to a state of 'aliveness' or 'culturability' *via* processes referred to as resuscitation. Thus, by definition, dormant cells are resuscitable, but this necessarily operational definition means that we can only tell that they were dormant, not that they are dormant.<sup>2,3</sup> Indeed, the ability to undergo dormancy (as with pheromone production<sup>37</sup>) is increasingly being recognised as an adaptive phenotypic trait (*e.g.* ref. 38–41). Ewald in particular (*e.g.* ref. 42–44) has stressed the evolutionary aspects of infectious diseases.

### Evidence

The evidence for a dormant blood microbiome comes mainly<sup>4,5</sup> from its direct assessment using culture-independent methods (although we know of Domingue's resuscitation papers<sup>45–47</sup>). Culture-independent methods include the detection of blood

(or tissue) microbial macromolecules such as rDNA<sup>48–59</sup> and the direct visualisation of cells using ultramicroscopic methods (*e.g.* ref. 5, 43, 45, 46, 60–64). In particular, it is recognised that dormant blood bacteria could 'hide' (or at least survive<sup>65–68</sup>) not only in white cells (*e.g.* ref. 69) but also within the (more than 1000-fold more numerous) erythrocytes. The significance of this, of course, is the sheer numbers that may be involved. If only one in 50 000 erythrocytes (that are present in blood at *ca.*  $5 \times 10^9 \text{ mL}^{-1}$ ) each harboured just a single dormant bacterium, there would still be more than  $10^5 \text{ mL}^{-1}$  (a number equivalent in urine to the threshold normally given for culturable cells in defining clinical bacteriuria).

Although there is evidence that a surprisingly large variety of bacteria can invade erythrocytes,<sup>5</sup> we know next to nothing about how they enter and egress from such cells. Even whether the latter involves pore-forming toxins<sup>70,71</sup> or haemolysins<sup>72</sup> that may effect membrane destruction, is unclear.

### Origins, and translocation into blood

We also rehearsed<sup>5</sup> the considerable evidence that minor leakages from the gut microbiome (*e.g.* ref. 73–75), even if only a tiny fraction of the 10–100 trillion<sup>76</sup> cells involved, whether *via* specialised structures such as M cells or more significant breaches in the gut epithelium (as can also occur in some cancers<sup>77</sup> and stroke<sup>78</sup>), are more than sufficient to provide a continuing inoculum to the bloodstream. Clearly the innate and adaptive immune system will normally remove those organisms threatening growth and noticeable bacteraemia, but this statement does not account for the fraction that become dormant and hide therefrom (whether geographically in cells and/or by losing their immunogenic potential, for instance by creating L-forms<sup>64</sup>). While the dormant bacteria do seem mainly to be hidden inside cells, their inflammatory products may not be. In the blood, LPS is typically bound either to an LPS binding protein (LBP)<sup>79–82</sup> which is a glycoprotein with a molecular weight of some 58 kDa<sup>83</sup> (452 amino acids<sup>84</sup>) or to the lipoprotein ApoE that is protective against LPS.<sup>85–87</sup> The ApoE4 polymorphism is of course well known as a genetic



locus favouring the development of Alzheimer's disease (e.g. ref. 87–92).

## 'Concentrations' of LPS and LPS-binding protein in blood

Our central argument is that low grade inflammation is mainly effected *via* the continuing production and shedding of LPS (and similar molecules) as dormant bacteria periodically awaken, proliferate and produce LPS before returning to dormancy. If this is going to be true, it is instructive to determine how much LPS and related molecules are typically found in human blood under various conditions. The potential load of LPS in the alimentary canal is  $\sim 1$  g.<sup>93</sup> We note, of course, that (as with serum ferritin<sup>8</sup>) the basis of these assays used to estimate concentration is typically a binding reaction, whether to an antibody or (in the case of LPS) based on a *Limulus* amoebocyte lysate (or its recombinant factor C<sup>94</sup>). Thus these and other (e.g. ref. 95–97) assays typically measure the (thermodynamically active) free forms, while the total amounts may be very much greater if (as with LPS) they are mainly bound to LBP or ApoE of HDL/cholesterol, or even monocyte surfaces.<sup>98</sup> Indeed, given that HDL-cholesterol is capable of sequestering LPS<sup>99</sup> (and lipoteichoic acid<sup>100</sup>), it is not surprising that there is considerable evidence that HDL-cholesterol is protective against sepsis and sepsis-related death,<sup>83,101–107</sup> showing further the importance of free LPS levels in disease prognosis.

This said, it is important to point out that if a substance is hydrophobic, *i.e.* poorly water-soluble, and its targets are hydrophobic (*i.e.* insoluble) as well, its measured potency also depends on the concentration of the hydrophobic elements containing the target (or otherwise).<sup>108,109</sup> In such circumstances, it is arguably better to speak of functional concentrations in terms of nmol per nmol target or similar, rather than in concentration terms (e.g. nM). In a similar vein, when considering properties such as cell death, what matters is the distribution of ligands between targets and the fraction of cells that die. In other words, if an added toxic molecule kills a cell (*i.e.* irreversibly) then this is a quantised property of the molecule, and again 'concentrations' are not an entirely meaningful manner with which to describe the toxic stimulus.<sup>3,110,111</sup>

Our main purpose here, though, is comparative, and aimed at obtaining a feel for the typical concentrations in health and disease, and those that are used in research studies. Note that as well as coming from infections, LPS is a common component of dust.<sup>112</sup> Thus, reported LPS 'concentrations' in healthy subjects seem to be of the order of 10–15 ng L<sup>-1</sup>, while those of LBP are roughly 1 000 000 times greater at 5–15 mg L<sup>-1</sup> (with both values increasing during sepsis) (Tables 1 and 2). LPS challenges of 5–100 µg per patient are commonly administered as experimental challenges and seen as 'safe'.<sup>112</sup> The larger volume, if distributed in 5 L of blood (a typical human value) equates to 20 000 ng L<sup>-1</sup>, which is obviously much higher than those free amounts typically measured even in sepsis. In terms of relating LPS to microbial biomass (see ref. 113),

**Table 1** A summary of LBP (LPS-binding protein) concentrations in health and disease

Tissue type	LBP in disease (mg L <sup>-1</sup> )	LBP in control (mg L <sup>-1</sup> )	Place	Ref.
Bacterial gastrointestinal infections	28.5 ± 16.5	—	Serum	122
Crohn's disease (CD) and ulcerative colitis (UC)	57.11 (49.4–65.8)	50.01 (37.1–63.9)	Plasma	123
Diabetes type 2	19.78 ± 6.40	20.53 ± 6.99	Serum	124
Endocarditis				
Infectious endocarditis	Median 33.41	Median 5.61	Serum	125
Noninfectious heart valve diseases	Median 6.67			
Inflammatory bowel disease	52.7 (45.4–64.6)	39.1 (32.1–43.7)	Serum	123
Lifestyle factors				
Smoking	7.11 (5.85–8.74)	7.18 (5.42–9.15)	Serum	83
Obese	5.90 (5.09–7.67)	7.75 (6.35–9.47)	Serum	
Overweight	5.90 (5.09–7.67)	7.29 (5.96–8.78)	Serum	
Metabolic syndrome	6.82 (5.48–8.40)	8.02 (6.63–9.82)	Serum	
Obesity, T2D and metabolic syndrome	27	10	Plasma	126
Liver				
Hepatocytes	5 to 15	—	Cells	127
Hepatic macrophages	LPS concentration were 10-fold higher than in the healthy controls	—	Plasma	128
Urinary tract infection in childhood	> 43.23	—	Serum	129
Sepsis				
Sepsis/septic shock LBP concentration at onset of severe sepsis	46.2 (3.74–155)	7.94	Serum	127
Sepsis in neonates	Median 36.6	Median 7.8	Plasma	130
Late-onset neonatal sepsis (LONS)	17.5	Unstated	Plasma	131
Gram +ve or Gram -ve sepsis	216	16	Plasma	132
(Higher in survivors)	31	4	Plasma	133
Septic shock tests	200	5–15	Unstated	134
Non-survivors 121 vs. 77 at 48 h	116–132 baseline		Serum	135
Remained much higher in non-survivors	34–55	8–15	Serum	136



**Table 2** A compilation of LPS levels observed in health and disease. An accepted conversion factor between endotoxin units (EU) and ng LPS is 1 ng endotoxin (LPS) = 10 EU

Disease	LPS in disease (ng L <sup>-1</sup> unless indicated as EU mL <sup>-1</sup> )	LPS in controls (ng L <sup>-1</sup> unless indicated as EU mL <sup>-1</sup> )	Tissue type	Ref.
Healthy individuals	—	0.15 to 0.35 EU mL <sup>-1</sup>	Plasma	137
Non-obese, post-menopausal women		10–20	Serum	93
Healthy controls		5	Plasma	133
HIV infection	60			138
Inflammatory bowel disease	12.6 (5.9–16.2)	12.2 (3.8–26.3)	Serum	123
Non-alcoholic fatty liver disease	7.8–14.8 EU mL <sup>-1</sup>	3.2–5.2 EU mL <sup>-1</sup>	Serum	139
Sepsis				
	300	7.3	Plasma	133
	470	Not noted	Whole blood	140
Type 1 diabetes				
Microalbuminuria group	31–60 EU mL <sup>-1</sup>		Plasma LAL assay	141
Normoalbuminuric group	38–74 EU mL <sup>-1</sup>		Plasma LAL assay	
Type 2 diabetes				
Non-obese postmenopausal women	—	0.37 ± 0.02 EU mL <sup>-1</sup>	Plasma LAL assay	93
Diabetic non-obese postmenopausal women	0.39 ± 0.03 EU mL <sup>-1</sup>		Plasma LAL assay	
Insulin-treated diabetes	6.6–10.7 EU mL <sup>-1</sup>	3.1–5.1 EU mL <sup>-1</sup>	Serum	142
Atherosclerosis	Above 50 gave 3 × greater chance of atherosclerosis	14		143

Watson and colleagues<sup>114</sup> showed in laboratory cultures that LPS amounted to some 50 fg cell<sup>-1</sup> in a logarithmic growth phase, falling to 29 fg cell<sup>-1</sup> in stationary phase, but in the oligotrophic conditions of seawater was just some 2.8 fg cell<sup>-1</sup>. This shows at once that LPS contents per cell can be quite variable, and that bacteria can shed a considerable amount of LPS at no major harm to themselves. On the basis that 1 mg dry weight of bacteria is about 10<sup>9</sup> cells, each cell is about 1 pg, so 50 fg LPS per cell equates to about 5% of its dry weight, a reasonable and self-consistent figure for approximate calculations. To deal with the fact that LPS is typically not a molecularly defined substance, its activity is sometimes reported in ‘endotoxin units’ (EU) based on a standard taken<sup>115</sup> from an *E. coli* O55:B5 strain; an approximate relationship is that 1 ng endotoxin ~10 EU. While the *Limulus* amoebocyte lysate assay is widely and effectively used as a test for pyrogens in parenteral solutions, its use in the estimation of LPS in blood is not considered especially reliable,<sup>116–120</sup> and it may be better to look more closely at LBP. This said, it is LPS that is the stimulus, and thus knowing its effective concentration is important. Unfortunately (Table 2), although the values in sepsis are considerably greater than are those in controls, there is a rather substantial variation between different studies, likely reflecting the rather different qualities of the assays used, the variation in the nature of the LPS (which is not a molecular entity), and the fact that much of the (rather hydrophobic) LPS *in vivo* is bound to other substances such that the result of the assay depends in significant measure on the extent and nature of any pre-extraction methods employed. Indeed, there are surprisingly few measurements of LPS in non-infectious low-grade inflammation, and very little evidence that plasma or serum LPS might be a particularly useful marker of it. The situation is a little clearer with LPS-binding protein (LBP), with a much more obvious distinction between controls and those

with sepsis. Less severe instances of infection include a median of 16 mg L<sup>-1</sup> for cirrhosis (interestingly reversed by the antibiotic norfloxacin).<sup>121</sup> Thus on the basis of present assay methods, there seems little benefit of seeking to follow the behaviour of a dormant blood microbiome with LPS measurements.

## A dormant tissue microbiome

Our previous reviews<sup>4,5</sup> (and many other works, *e.g.* those summarised in ref. 43, 62, 64 and 144) outlined in some detail the fact that many known infectious agents can enter cells and persist intracellularly, and those discussions are not repeated in detail here. Indeed, the very existence of eukaryotes is considered to be based on the intracellular uptake of prokaryotes to form structures such as mitochondria,<sup>145</sup> and there is increasing evidence for dinitrogen fixation by endosymbionts in plant leaf cells (*e.g.* ref. 146 and 147). Regarding human tissue, as Nash and colleagues put it,<sup>144</sup> “the blood is the most effective vehicle of all for the spread of microbes through the body. After entering the blood they can be transported within a minute or two to a vascular bed in any part of the body. In small vessel such as capillaries and sinusoids where blood flows slowly, there is an opportunity for the microorganism to be arrested and to establish infection in neighbouring tissues.” (The same holds true, of course, for circulating tumour cells and their role in metastasis.) Later we shall look at this translocation from blood to tissues in more detail. However, we first mention an example that we did not deal with previously in much detail, *viz.* *E. coli*-based cystitis.

### *E. coli*-based cystitis

Cystitis (inflammation of the bladder) is commonly caused by urinary tract infection, typically by *E. coli*,<sup>148,149</sup> and



especially in women. It can also lead to bacteraemia.<sup>150</sup> A particular point of present interest regarding dormancy<sup>151</sup> is the fact that a high percentage of cystitis patients suffer reinfection,<sup>152–159</sup> that is often clearly from the same strains that caused the original infection.<sup>160–164</sup> This has led to the recognition in bladder epithelial cells of so-called ‘quiescent intracellular reservoirs’<sup>156,158,165–170</sup> of dormant cells that can resuscitate. Because one cannot determine these things in humans *in vivo*, it is not known precisely how they enter such cells after binding to appropriate receptors such as uroplakins,<sup>171</sup> but it is presumed that as with many other cells where it is better understood this occurs *via* endocytosis of some kind.

Separating the blood from certain tissues are physical barriers such as the blood–brain, blood–retina and blood–testis barriers, consisting of layers of epithelial cells with especially tight junction. They are of notable significance to drug transport(ers) as well.<sup>172–175</sup> It is of particular interest that even here we can find that these barriers are (or must be) breached from time to time, as dormant microbes can be found even in the CNS. Possible means of resuscitation are discussed below and elsewhere.<sup>4</sup>

## A dormant CNS microbiome

As one might suppose, the CNS differs little from other tissues with regard to the possibility that dormant microbes may persist there, occasionally ‘waking up’ to cause trouble. Three examples, as they pertain to the aetiology of Alzheimer’s disease (and presumably other dementias) are represented by *Chlamydia pneumoniae* (as stressed by Balin and colleagues, *e.g.* ref. 176–185), by herpes simplex virus (as highlighted by Itzhaki and colleagues<sup>30,88,182,186–195</sup>) and by a variety of spirochetes (as championed by Miklosy and colleagues<sup>196–203</sup>). The latter is consistent with the well-established dementia in the terminal stages of another spirochetal disease in the form of syphilis, and also with Lyme disease.<sup>199,204–206</sup> Of course multiple classes of microorganisms may contribute. There is also evidence for a CNS involvement of the parasitic protozoan *Toxoplasma gondii* in a numbers of neurodegenerative diseases.<sup>207–209</sup>

The ‘gut-brain axis’ describes the well-established observations of a bidirectional neurohumoral communication system in the human body. Given the above, it is not surprising that there has been increasing recognition over the last couple of years of quite overt (and bidirectional) communication between the gut microbiome and the CNS *via* the gut-brain axis, in particular, the idea that bacteria in the gastrointestinal (GI) tract can activate neural pathways and CNS signalling systems, from the earliest moments in life. Dysfunctions of this axis can lead to all kinds of neurological problems, including anxiety, depression and other CNS disorders (*e.g.* ref. 210–229). Most studies have focussed on the endocrine and immune systems. As yet, however, we have found no literature that has sought a role for LPS here, although given that LPS is actually used in a variety of experimental models (*e.g.* for Parkinson’s<sup>4,230–237</sup> and even obesity<sup>238</sup>) to initiate CNS disorders, it is an easy

prediction that it is likely to play a significant role in the gut microbiota–brain interaction.

## A high-level overview of the effects of LPS in sepsis, septic shock and SIRS

Although our focus here is more on chronic inflammatory states induced by dormant and resuscitating bacteria, it is instructive first to consider events that occur in the more extreme and life-threatening cases of sepsis, septic shock, and the systemic inflammatory response syndrome (SIRS). While these are commonly observed in the Intensive Therapy Unit as a result of an initial infection (hence the term ‘sepsis’), their typical treatment there with broad spectrum antibiotics means that proliferating microbes are rare or absent, and it is their products such as LPS that are then the main problem. Specifically, although these are responsible for invoking the innate immune response that triggers cells to attack and dispose of the invading microbes, an overstimulation of these activities leads to the life-threatening ‘cytokine storms’ that are the proximate causes of, and reflect, endotoxic or septic shock or SIRS (*e.g.* ref. 7 and 239–247).

It is worth rehearsing the definitions<sup>248,249</sup> to help discriminate sepsis<sup>250,251</sup> from its sequelae. Thus, sepsis has been defined as “the presence (probable or documented) of infection together with systemic manifestations of infection”,<sup>252</sup> while severe sepsis is defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion<sup>252</sup> Septic shock is “sepsis-induced hypotension persisting despite adequate fluid resuscitation”.<sup>252</sup> SIRS refers to “the systemic inflammatory response to a variety of severe clinical insults” (infectious or otherwise). It usually involves two or more of the following criteria: (i) temperature > 38 °C or < 36 °C; (ii) heart rate > 90 beats per m; (iii) respiratory rate > 20 breaths per m or PaCO<sub>2</sub> < 32 mm Hg; (iv) WBC count > 12 000 μL<sup>-1</sup> or < 4000 μL<sup>-1</sup> or (v) > 10% immature neutrophil forms (*i.e.*, “bands”).<sup>253,254</sup> The chief point about recognising and using SIRS instead of ‘sepsis’ is, of course, that it does not rely on the presence of observable (or culturable) microbes (and it can anyway be caused by traumas lacking an immediate microbial component). A Venn diagram (redrawn from ref. 254) illustrates the main ideas (Fig. 3).

Clearly any increases in microbial cell numbers increase the likelihood of LPS production and shedding that leads to the cytokine storm. Thus, the progression of the microbial variant in unfavourable cases goes roughly from left to right in Fig. 4, as infection → bacteraemia → LPS → sepsis → septic shock → SIRS → multiple organ failure (MOF/MODS) → death. Mortality rates from sepsis/SIRS are extremely high (30–70% in intensive care units),<sup>241,242</sup> and dependent on age.<sup>255</sup> Note too that antibiotics can themselves promote shedding of LPS from dying bacteria (*e.g.* ref. 118 and 256–265), especially from spirochetes such as *Borrelia burgdorferi*, leading to a Jarisch–Herxheimer (JH) reaction.<sup>266,267</sup> The JH reaction can be mitigated by antibodies to TNF-α.<sup>268</sup> Importantly, this continual shedding of LPS is a normal property of growing Gram-negative bacteria, especially in



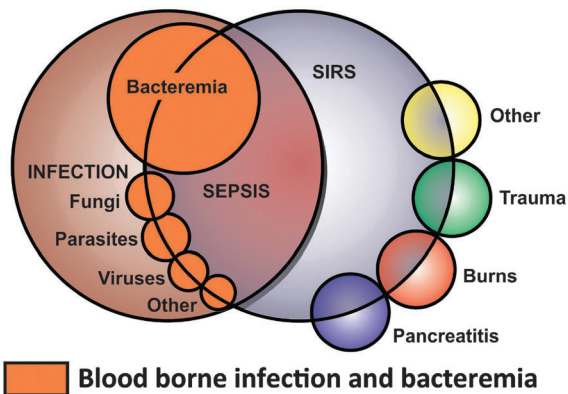


Fig. 3 Relationships and overlaps between bacteraemia, sepsis and systemic inflammatory response syndrome. Redrawn from ref. 254.

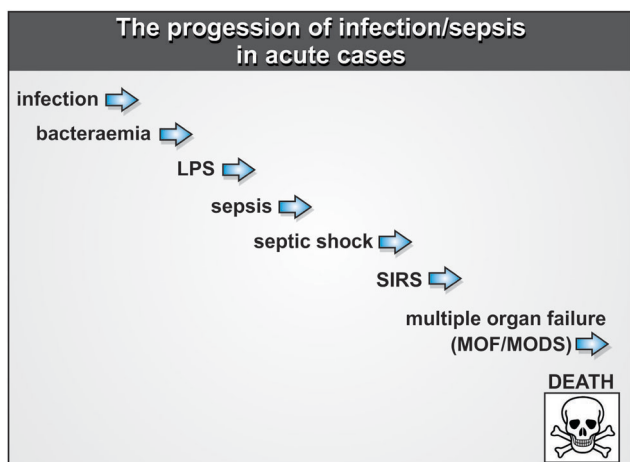


Fig. 4 The main steps that represent the progression of infection/sepsis in acute cases.

media such as those containing serum that are inimical to their growth.<sup>257,269</sup> This has obvious implications.

Even if one survives septic shock, there are other sequelae, such as long-term cognitive impairment,<sup>270</sup> cardiovascular<sup>271</sup> and other<sup>272</sup> complications that may reflect or contribute to symptoms seen under more chronic conditions.<sup>273</sup> Indeed, there are a great many comorbidities between various diseases with a microbial component.<sup>36</sup>

Clearly, LPS plays a central role in the development of inflammation. We therefore spend time in the next paragraphs to discuss the role of LPS in the cellular inflammatory processes.

## Positive feedback and amplification in integrative and systems biology

The first point to make here is a general one about how various kinds of kinetic schemes or network topologies can amplify biochemical signals. For a single enzyme, if its product or any other molecule is an uncompetitive inhibitor that binds only to

the enzyme–substrate complex, a small amount of this can lead to a very large increase in a substrate concentration. This serves to explain both the peculiar effectiveness of glyphosate as a herbicide,<sup>274–277</sup> and the extreme rarity of uncompetitive inhibition in natural systems.<sup>278</sup> One type of network-based amplification, that is familiar in signalling cascades, is one in which a signalling activity such as a kinase changes the activity of another kinase, and so on. Here, as well as amplification, the cascade is partly about serving as a suitable delay loop,<sup>279</sup> as clearly variations in amino acid sequence can and do<sup>280</sup> have major effects on the activities of individual proteins such that the cascade would otherwise seem unnecessary. A second kind of amplification, known as ultrasensitivity, comes from a structure in which an effector stimulates by covalent modification (e.g. phosphorylation) of an enzyme catalysing a particular reaction, while simultaneously inhibiting a second enzyme (e.g. a phosphatase) catalysing the removal of the covalent modification. This leads to very large changes in flux and network behaviour as the concentration of the effector passes a threshold.<sup>281–285</sup> Similarly, pulsatile or oscillatory signals can be much more effective for the same ‘average’ concentration.<sup>286,287</sup> Thus, a variety of network motifs can provide ‘sniffers, buzzers, toggles and blinkers’.<sup>288</sup> And most of all, although other behaviours are possible,<sup>289</sup> a variety of simple systems with positive feedback can amplify a very small signal into a much larger one (Fig. 5). This can typically occur in inflammatory systems<sup>7</sup> where molecules whose production is induced by LPS, such as IL-1 $\beta$ <sup>290–293</sup> (Fig. 6) or TNF $\alpha$  (see later), can stimulate their own synthesis or effect crosstalk (e.g. IL-1 induced TNF- $\alpha$ <sup>294</sup>).

A second major area where an effector can appear to amplify a small stimulus, or have a large effect, is when it interacts with multiple targets simultaneously (in drug discovery this is known as polypharmacology (e.g. ref. 295–297)). This need actually follows from the principles of systems biology as encapsulated in metabolic control analysis,<sup>298–301</sup> where mediators that modify only one target can rarely be expected to have much effect. As we shall see, LPS qualifies here too, as it stimulates a great many proinflammatory and proapoptotic pathways, including those necessary for its translocation in both free and bacterial cell-associated forms.

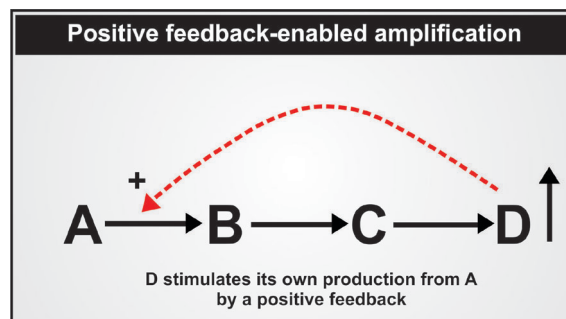


Fig. 5 A system exhibiting positive feedback in which a product stimulates its own synthesis. In a biochemical context, A to D represent metabolites, while the arrows represent enzymatic steps.



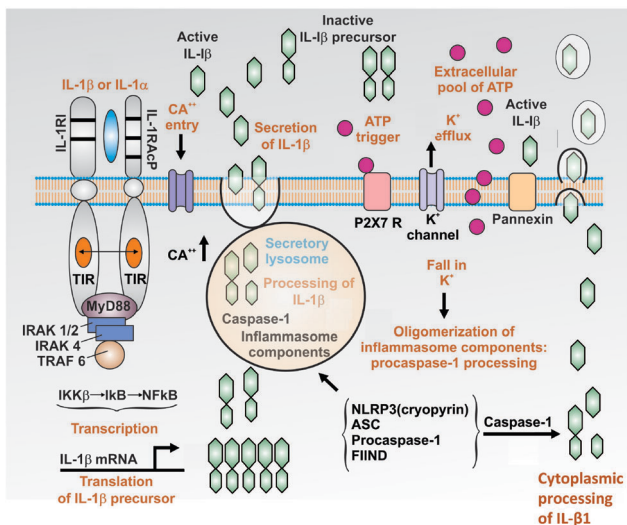


Fig. 6 IL-1 $\beta$  is an example of a cytokine that can stimulate its own synthesis (figure redrawn from and based on one in ref. 291).

Thus we shall see that LPS is likely to serve as a major nexus in inflammation in general and that induced by microbes in particular.

## Structure and function of LPS

An overall 'cartoon'-type structure of typical LPS molecules is given in Fig. 7A based on.<sup>302</sup> They consist of an inner lipid A core and a number of branched polysaccharide chains (*e.g.* ref. 302–307), terminating in those that determine the strain's serology *via* the O-antigen.<sup>308</sup> The biosynthesis is discussed by Wang and Quinn.<sup>309</sup> According to the comprehensive LIPID-MAPS classification,<sup>310</sup> LPS is a saccharolipid glycan. The lipid A core is significantly the most inflammatory part of the molecule,<sup>311</sup> with typically two *N*-acetylglucosamine residues attached to a 2-keto-3-deoxy-*D*-manno-octulosonic acid (Kdo) disaccharide (Fig. 7B). Bacteria lacking the outer O-antigen chains are known as 'rough' and are significantly more immunostimulatory than are their 'smooth' equivalents that contain them.<sup>312</sup>

## A systems perspective on LPS-induced cytokine production and cell death

A standard approach to systems biology modelling (*e.g.* ref. 279 and 289) has four main stages. The first two are qualitative, and involve determining the players and how they interact, whether as substrates, products or effectors – this establishes the topology of the network. The second two are more quantitative, involving the mathematical form of the equations describing each step, and their parametrisation, also involving the running of the model, typically as a set of coupled ordinary differential equations, using suitable software (*e.g.* ref. 313–315). Note too that in a typical network of this type, a systems approach typically discriminates parameters from variables. Parameters are either fixed or outside the control of the experimenter; they

typically include the unchanging concentrations of substances involved in flux-generating steps, as well as kinetic constants such as  $K_m$  and  $k_{cat}$ . By contrast, variables are those things that vary during an experiment, typically involving concentrations of intermediary substances and fluxes through pathways towards 'exit' variables. Thus, figures such as those in Fig. 1, while accurate in the sense of illustrating flows of information, are misleading because they are at once both static and qualitative. While we can and shall point to many papers that show clearly that "LPS can cause inflammation (or apoptosis)", such a statement too is less than complete. This is because individual papers rarely if ever state, use or vary systematically a number of parameters that are known to have a huge impact on cell fate. These include

- The exact type of LPS (even though some, especially their lipid A component, are known to be much more immunogenic or inflammatory than are others – see *e.g.* ref. 306, 309 and 316–324 and later)
- The rather variable amount of LPS added, whether the assay is for cytokine production (Table 3) or 'viability' (Table 4)
- The nature of the host (human or rodent, that respond differently,<sup>325–327</sup> or *in vitro*)
- Whether the LPS is added in a bolus or a dynamic manner (this matters a lot<sup>287</sup>)
- Whether measurements are done in single cells or as an ensemble, and or quasi-continuously; this matters because much evidence in the NF- $\kappa$ B system<sup>287,328–330</sup> and other related systems<sup>331–334</sup> shows that it is the nature and dynamics of the oscillations that determines which genes are transcribed and with which kinetics, as well as cell fate, and not just say an NF- $\kappa$ B concentration at a particular time. Thus knowledge of single-cell behaviour is vital<sup>3,110,111</sup> (also in pharmaceutical drug uptake<sup>174,175</sup>).
- Which other substances, conditions or parameters have been co-varied or even recorded, and whether they themselves are known to modify the effect of LPS alone. One example is ATP, which activates the purinergic PX27 receptors and increases massively the extent of cell death.<sup>335,336</sup> Another is acidosis.<sup>337</sup>

## LPS as a stimulant of inflammatory cytokine production: cellular mechanisms

While only partly consistent with the 'danger theory' of the immune response,<sup>364,365</sup> and more obviously stemming from the ideas of Janeway,<sup>366</sup> LPS is recognised as a major 'pathogen-associated molecular pattern' or PAMP that triggers the body's innate immune response to pathogens (*e.g.* ref. 367). In addition, cells release damage-associated molecular pattern molecules (DAMPs) as signals that alert the innate immune system to unexpected cell death and to microbial invasion. It is now very well established that a chief means by which LPS excites an inflammatory innate immune response is by binding to the toll-like receptor 4 (TLR4).<sup>368–373</sup> Typically, the LPS is bound in





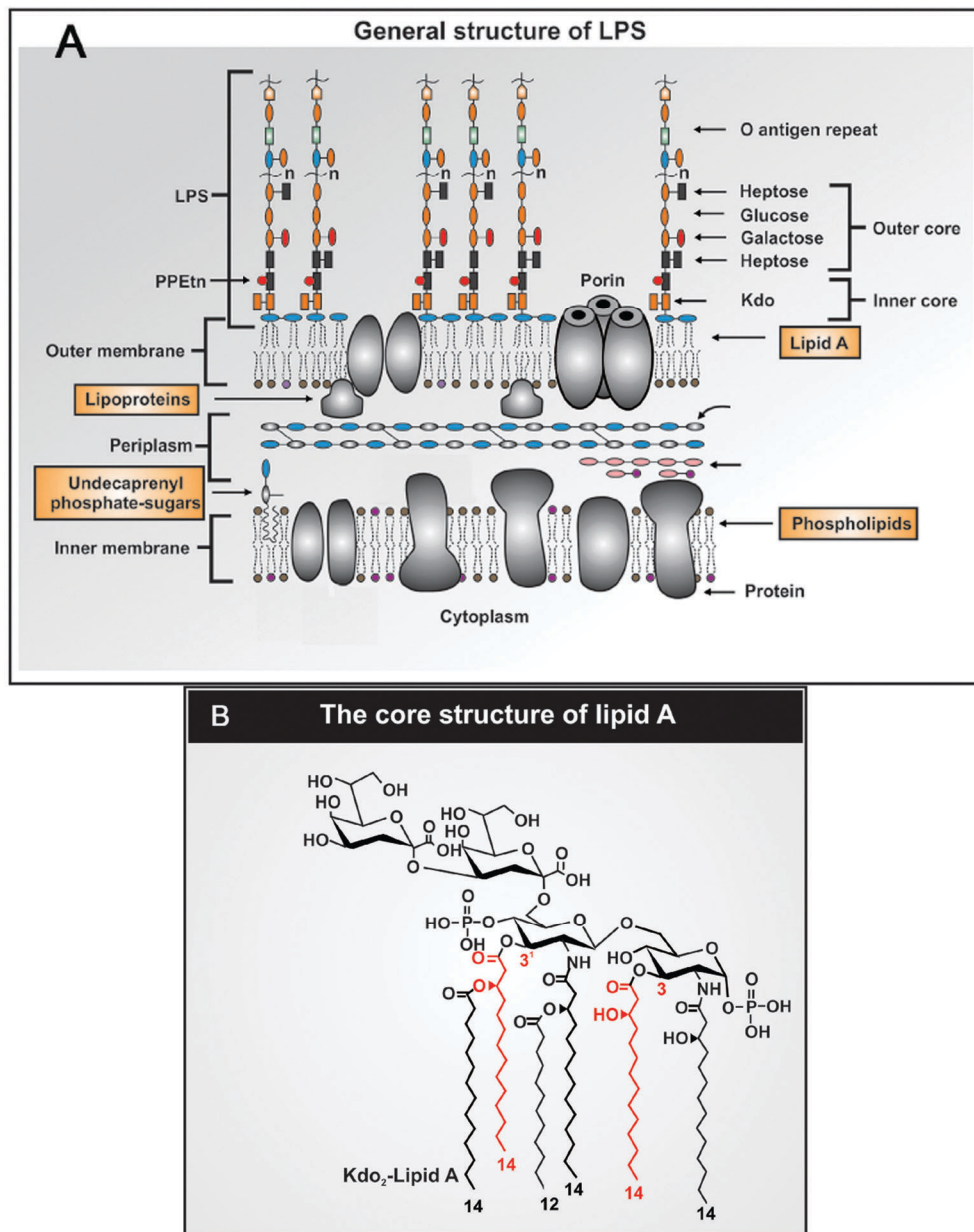


Fig. 7 The general structure of (A) LPS and (B) its core lipid A component. Redrawn from ref. 302.

blood to LBP, and the LPS is ferried to the TLR4 receptor *via* a CD14 co-receptor (which also links innate immunity with Alzheimer's disease<sup>374–377</sup>). This binding of LPS to TLR4 in turn activates the production of a variety of pro-inflammatory cytokines.<sup>378–381</sup> The extent of cytokine activation reflects in part the strength of binding to CD14/TLR4.<sup>320</sup> These inflammatory cytokines are induced *via* a set of canonical pathways illustrated in Fig. 8, with the transcription factor NF- $\kappa$ B playing a prominent role.<sup>382–385</sup> As is also well known (and see below), NF- $\kappa$ B is normally held inactive in the cytoplasm by being bound to an inhibitor I $\kappa$ B protein, and the means by which extracellular signals such as LPS are transduced involve a series of kinases, one of which (IKK) in particular phosphorylates the I $\kappa$ B and thereby releases the NF- $\kappa$ B that can translocate to the

nucleus to turn on a large variety of other genes, including in particular TNF- $\alpha$  and IL-6.<sup>386</sup> There is also a 'non-canonical' inflammasome LPS activation pathway independent of TLR4,<sup>387–389</sup> that occurs at higher external concentrations of LPS,<sup>23,390</sup> comes into play when the LPS is internalised, and involves (*via* p38 MAP kinase and intracellular LPS) the activation and secretion of cytokines such as IL-1 $\beta$  (Fig. 9) and also TNF- $\alpha$ .

## Muropeptides and host signalling

A more recent recognition is that as well as lipid A, shorter bacterial cell-wall derived muropeptides also have a significant role in innate immunity (*e.g.* ref. 392–405; they act synergistically



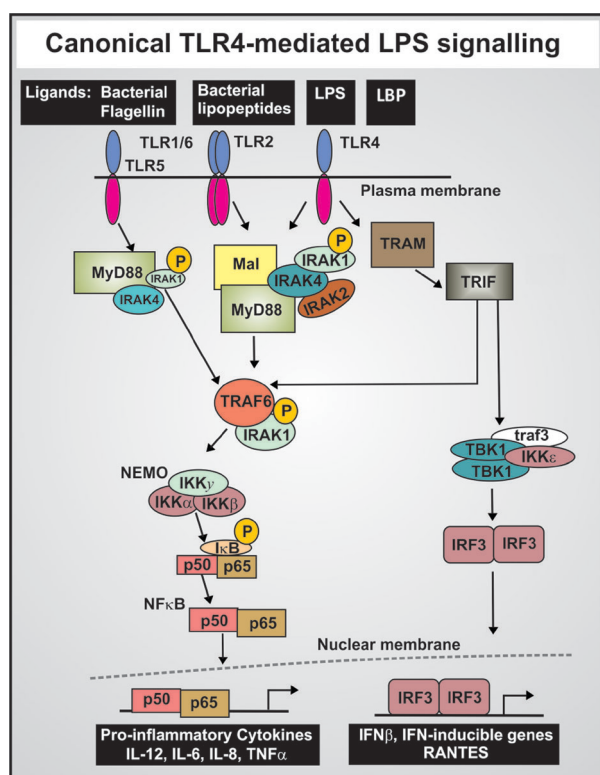


**Table 3** Some examples of LPS administered to cells (primary or permanent cell culture or *in vivo*) or animals and its effect on interleukin and TNF- $\alpha$  production

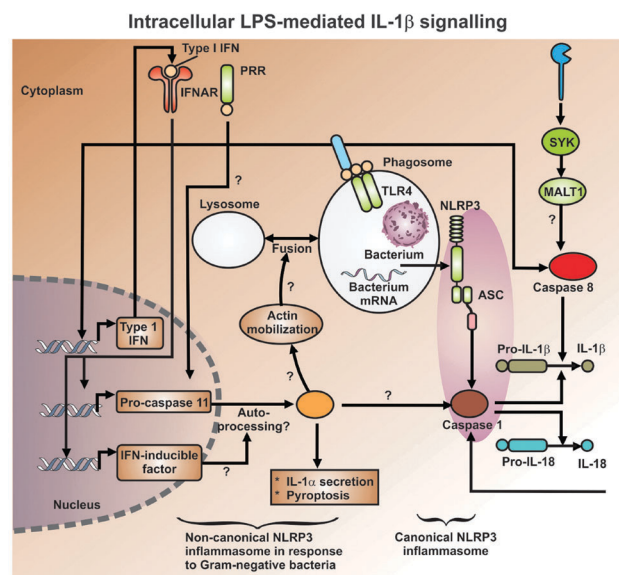
Cell type/animal origin	Cell name	LPS concn administered to cells/animals	LPS type	Measurement: interleukin and TNF- $\alpha$ production	Ref.
Mouse origin					
Peritoneal mouse macrophages	Raw 264.7 mouse macrophages	100 ng mL <sup>-1</sup>	<i>Escherichia coli</i> O55:B5	Increased IL-6 production	338
C57BL/6 mice	Primary microglial cultures	100 ng mL <sup>-1</sup>	<i>E. coli</i> O26:B6	Massive increase in IL-1 $\beta$ when purinergic receptor activated	335
IL-1Ra knockout (KO) and wild-type (WT) mice	Peritoneal macrophages (primary cultures)	10 $\mu$ g mL <sup>-1</sup>	<i>Aggregatibacter actinomycetemcomitans</i> LPS	Increased production of IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ and IL-6 in IL-1Ra KO mice, compared with the levels in WT mice	339
Mouse alveolar macrophages	MH-S cell lines	100 ng mL <sup>-1</sup>	Sigma LPS type not stated	LPS stimulated overproduction of TNF- $\alpha$ and IL-10	340
C57BL/6 mouse	Primary macrophages	1 $\mu$ g mL <sup>-1</sup>	<i>E. coli</i> O26:B6	Massive increase in IL-1 $\beta$ but not IL-6	341
Mouse microglia	BV2 microglia	100 ng mL <sup>-1</sup>	<i>E. coli</i> O55:B5	LPS-stimulation induces the expression of inducible NOS (iNOS) and COX-2	342
Intact mice ( <i>ob/ob</i> ), or C57BL/6 with diet-induced obesity		5 $\mu$ g or 100 $\mu$ g kg <sup>-1</sup>	<i>E. coli</i> O127:B8	Massive increases in IL-1 $\beta$ , IL-6 and IL-RA	238
J774.A1 macrophages	Primary cultures	10 $\mu$ g mL <sup>-1</sup>	Not stated, but was <i>E. coli</i> O26:B6	Huge increase in IL-1 $\beta$ production	343
Rat origin					
Rats	Serum level measurement	100 $\mu$ g per rat, ip	Not stated	Increased IL-6 and TNF- $\alpha$ production	344
Sprague-Dawley rats hippocampal brain slices	Primary culture	10 $\mu$ g mL <sup>-1</sup>	<i>E. coli</i> O55:B5	Significant increased concentration of TNF- $\alpha$ and IL-1 $\beta$	345
Human origin					
Human bone marrow aspirates	Bone marrow mesenchymal stem cell primary cultures	0 to 10 $\mu$ g mL <sup>-1</sup>	<i>Porphyromonas gingivalis</i> LPS	IL-6 production independent of the LPS dosage	346
Peripheral blood mononuclear cells	Primary culture white blood cells	0.1, 1 or 100 ng mL <sup>-1</sup>	Not stated	IL-6 and TNF- $\alpha$ were both strongly upregulated	347
HepG2	Hepatoma cell line	0.1, 1 or 100 ng mL <sup>-1</sup>	Not stated	Slight inhibition of TNF- $\alpha$	347
Peripheral blood mononuclear cells (PBMC) (from healthy and type 2 diabetes individuals (T2D))	Primary culture white blood cells	2 and 0.2 ng mL <sup>-1</sup>	TLR4 ligands LPS	With low LPS dose the T2D cohort exhibited enhanced IL-1 $\beta$ relative to healthy cells	348
Nasal polyplfibroblasts	Primary cultures	10 $\mu$ g mL <sup>-1</sup>	<i>E. coli</i> O111:B4	LPS enhanced the secretion of IL-6	349
Other animal origin					
Airway neutrophilia was induced in horses by inhalation of LPS	Animal model	1 mg mL <sup>-1</sup> per horse	Sigma LPS type not stated	Significant increased concentration of IL-6 and TNF- $\alpha$	350

**Table 4** Some examples of LPS administered to cells (primary or permanent cell culture or *in vivo*) and its effect on cell viability

Cell type	Cell name	LPS conc (ng mL <sup>-1</sup> )	LPS type	Viability assay	Viability %	Ref.
Rat origin						
Rat duodenum	Epithelial cells	0.75–3 mg kg <sup>-1</sup> i.v. or 3–12 mg kg <sup>-1</sup> p.o	<i>H. pylori</i> LPS	MTT	60%	351
Rat myocytes		25–10 000 (most 100)	Unstated	Apoptosis	80%	352
Alveolar macrophages	NR8383	10	Unstated	LDH and Hoechst/PI	90% without particulates	353
Myocardial myocytes	H9c2	1000		MTT, LDH, TUNEL, JC-1	85%	354
Myocardial myocytes	H9c2	20 000		MTT	65%	355
Mouse origin						
C57BL/6 mice	Primary microglial cultures	100	<i>E. coli</i> O26:B6	LDH release	~10% in presence of ATP	335
Osteoblast	MC3T3-E1	10 100 1000	<i>E. coli</i> O55:B5	MTT	70%	356
Mouse macrophages	RAW 264.7	100	Unstated	MTT	Not stated; increased apoptosis	357
Macrophages	BMDM	100 (6 h)	Unstated	DNA fragmentation	~60%	358
Human origin						
Pulmonary epithelia	A549	1000	Unstated Sigma	Unstated kit	60–70%	359
Human PBMC	PBMC	0.01–3	<i>E. coli</i> unstated	None – only IL-8 and ROS		360
Microglial	BV2	10 000–30 000	Unstated	MTT and trypan blue	~60%	361
Vascular human endothelia	HUVECs	500	Unstated	Annexin	80%	362
Dopaminergic	SH-SY5Y	100	<i>E. coli</i> O127:B8	MTT	Unclear	363

**Fig. 8** The LPS-mediated cellular production of inflammatory cytokines. Canonical pathway of LPS-mediated release and nuclear translocation of NF-κB (based on ref. 379).

with LPS,<sup>396</sup> probably because they also interact with the NF-κB pathway, *via* the RICK/Rip2/CARDIAK kinase<sup>406,407</sup>). It is of particular interest in the context of bacterial dormancy that such muropeptides seem to be part of the ‘wake-up’ activity of the

**Fig. 9** The intracellular LPS-mediated activation of caspase-1 leading to IL-1β production (after ref. 391).

bacterial Rpf<sup>408</sup> and other bacterial resuscitation systems.<sup>409</sup> See Fig. 10 for a visual representation of the host signalling pathway of MDPs.

## LPS induction of apoptotic, programmed necrotic and pyroptotic cell death

A particularly important inflammatory cytokine whose secretion is induced by NF-κB (and also by p38 MAP kinase) is



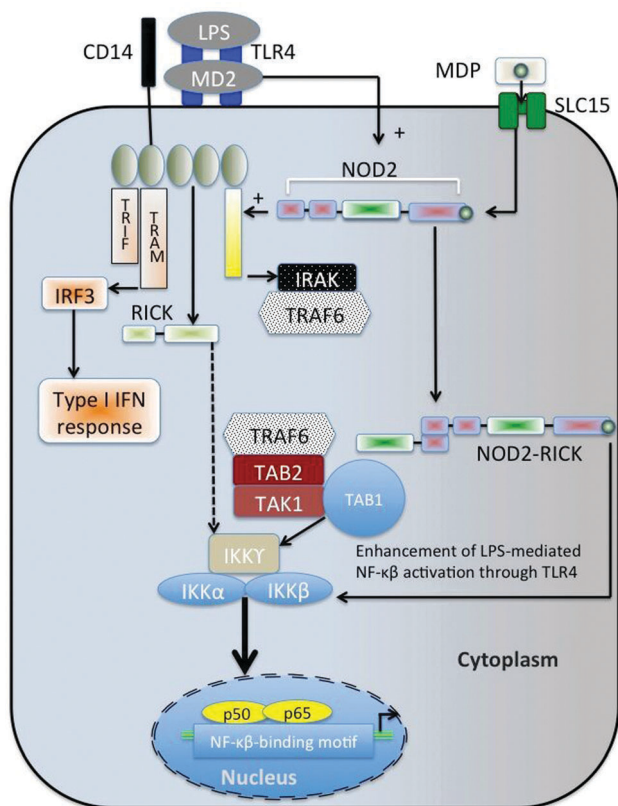


Fig. 10 Host signalling pathways of muramyl peptides (MDP). Based on and redrawn from ref. 405. MDP are taken up *via* the pepT1 oligopeptide transport system (SLC15 – see ref. 410).

TNF- $\alpha$  (*e.g.* ref. 411–414). This too is inflammatory and itself induces further changes in NF- $\kappa$ B expression. The same is true for IL-1 $\beta$ .<sup>343,378</sup> In particular, these inflammatory cytokines can lead to apoptotic cell death (*e.g.* ref. 343 and 358). As we shall see, however, and as presaged in Tables 3 and 4, it is quite difficult to establish precisely what is going on in many cases, as individual studies tend to study a restricted set of pathways and individual players, be they NF- $\kappa$ B, p38, IL-1 $\beta$  or IL-6. It would not in our view be completely unfair to describe a lot of these studies collectively as ‘a bit of a mess’. Taking NF- $\kappa$ B as a canonical example (though the same is true for p38<sup>415</sup>), what are ostensibly the same kinds of signal can lead to dramatically different cell fates, *e.g.* proliferation *vs.* apoptosis<sup>416–424</sup> depending on the conditions. What this is telling us, of course, since these processes are considered largely deterministic, is that we are not measuring or controlling all of the relevant factors (see also Table 3), and in great measure these studies are largely qualitative in nature. Here our purposes are thus simply to recognise that the genes induced or repressed *via* transcription factors such as NF- $\kappa$ B can be pro- or anti-apoptotic, and which ones are activated depend on all prevailing conditions.

As well as apoptosis, there is a less tightly (but partly) regulated form of cell death known as ‘programmed necrosis’ or ‘necroptosis’,<sup>425–437</sup> that may be induced by inflammatory

ligands such as TNF, especially during infection, and that sometimes also involve NF- $\kappa$ B. Another important mode of cell death induced by related stimuli is pyroptosis<sup>438–450</sup> (that involves the caspase 1-dependent production of IL-1 $\beta$ ). Ferroptosis<sup>451,452</sup> is a cell death mechanism that stresses the importance of unliganded iron (see also ref. 6, 7, 453 and 454). Although the stimulus in each of these cases is nominally the same (LPS of some kind) there are presumably pre-existing conditions that differ and thereby determine precisely the kind of cell death that ensues. However, we do not discuss the emerging differences in their molecular details nor taxonomy here, since the important thing for the present arguments is simply that the cells die, disappear, and thereby leave gaps where once they lived.

## LPS as a stimulant of coagulation and thrombosis

As we recently reviewed,<sup>9</sup> a hallmark of many chronic, inflammatory diseases is the fact that they simultaneously exhibit both hypercoagulability and hypofibrinolysis. While a great many biochemicals can influence both the kinetics and end-product structures of the clotting process, and we previously highlighted unliganded iron<sup>8,10,455–458</sup> and the fibrin concentration itself,<sup>9</sup> we can hardly avoid noting that LPS itself is a strong procoagulant.<sup>459–463</sup> How direct some of these mechanisms are seems not to have been established, though certainly LPS can bind to erythrocyte membranes.<sup>464,465</sup> Given that promiscuity correlates with hydrophobicity (*e.g.* ref. 173 and 466–469), it is not surprising that the very hydrophobic LPS can potentially interact with a great many (lipo)proteins; its ability to convert prions to their more toxic PrP<sup>Sc</sup> form<sup>470</sup> is a pertinent case in point.

## The role of microparticles and LPS

We wish, however, to spend some time on the possible involvement of LPS in microparticle formation. Microparticle formation is typically *via* apoptosis and the related pathways described above.<sup>471–474</sup> Such microparticle formation is prominently associated with inflammatory conditions. Microparticles have also been associated with proinflammatory effects and also with autoimmune processes, and they are thought to be a source of autoantigenic nuclear material, which can form immune complexes.<sup>475</sup> Various cells such as platelets, lymphocytes, endothelial cells, erythrocytes and monocytes do release surface-derived microparticles<sup>476</sup> and these microparticles are seen as multi-purpose carriers.<sup>477</sup> They carry proteins, lipids and nucleic acids, and play a fundamental role in the pathogenesis of thrombosis and are known to modulate the properties of target cells.<sup>478</sup> Microparticles from erythrocytes also carry heme and these heme-laden microparticles have a physiopathological impact on the rest of the haematological system.<sup>479</sup> Microparticles also frequently elicit an immune response.<sup>480–483</sup>

As microparticles are known to be present in many inflammatory diseases,<sup>8,478,484–487</sup> they might therefore develop *via* an



external or internal stimulus on cells (e.g. erythrocytes and platelets), and here we suggest that the stimulus might be LPS. While pure lipid systems are not always good membrane mimics,<sup>172–174</sup> LPS has been shown to insert spontaneously into lipid bilayers, and this insertion can lead to membrane breakdown.<sup>480,488,489</sup> This insertion capability has also been demonstrated in lipid raft models.<sup>490</sup> Given that we have recently shown that bacteria can hide inside erythrocytes, shedding of LPS may thus occur within the cells (as well as obviously outside the cells, where “free” bacteria may shed LPS). There is considerable literature that suggests that LPS can be a cause of apoptosis etc, so LPS shed from internalized

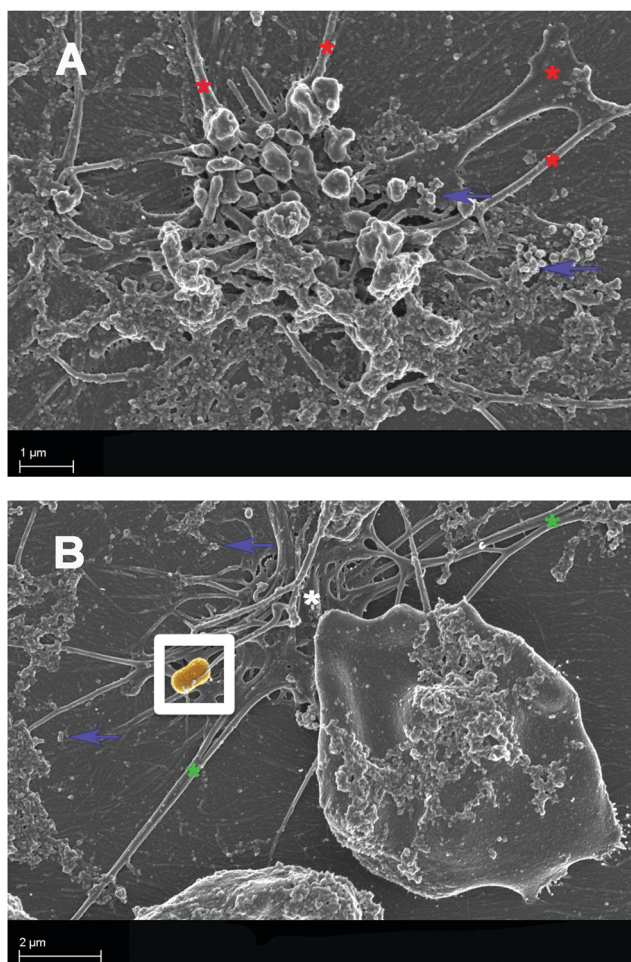
cellular bacteria may also trigger apoptotic pathways from within the cells (*via* caspase-11, caspase-4 and IL-1 $\beta$  pathways), resulting in microparticle formation. This eventually stimulates the processes of coagulation and thrombosis already known to be associated with microparticle presence in inflammatory conditions.<sup>491,492</sup> An example of this is shown in Fig. 11A and B, where microparticle formation in thrombo-embolic ischemic stroke is seen associated with both hyperactivated platelets and damaged erythrocytes, together with the presence of bacteria.

## The sequelae of intestinal inflammation and LPS-induced apoptosis, necroptosis and pyroptosis include epithelial permeabilisation

It is clear that as the concentration(s) or activities of LPS, inflammatory cytokines and other mediating factors increase, cell death is an inevitable consequence.<sup>425,493–495</sup> While, as mentioned above, there is almost certainly a continuing small leakage of microbes from the gut<sup>496</sup> (known as the ‘leaky gut’ hypothesis), it is evident that a variety of conditions, that we may loosely refer to as ‘stress’ can increase this considerably (e.g. ref. 73, 78 and 497–505). Bacterial sepsis itself is one such stress,<sup>506</sup> and interestingly there is now a burgeoning literature to the effect that the immunodeficiency seen in HIV/AIDS patients may actually be caused by gut-derived LPS causing hyperactivation (then death) of CD4<sup>+</sup> cells (e.g. ref. 138 and 507–509). There does not seem to be a major genetic contribution to leaky gut.<sup>510</sup> Although many of the same signalling pathways are involved, the extent to which this is mediated *via* LPS is not yet clear, albeit LPS itself can indeed disrupt tight junctions and increase intestinal permeability.<sup>511–516</sup> Overall, these kinds of endothelial dysfunction clearly lead to increased leakiness or permeabilisation<sup>430</sup> (Fig. 12).

## Localised microbial proliferation, inflammation and cell death as a cause of specific diseases

We have here sought to provide a rather general explanation for the role of LPS-induced chronic inflammation in a variety of different diseases, with the ‘continuing’ element driven by the resuscitation of a resident blood and tissue microbiome. However, it is obvious that while all these diseases are inflammatory (and have been linked to microbes<sup>4,5</sup> and/or LPS (Fig. 2), diseases such as Alzheimer’s, Parkinson’s, atherosclerosis and rheumatoid arthritis obviously occur – or one might better say manifest – in largely different tissue or tissue subtypes. Thus, the disappearance of cells from the CNS can manifest as Parkinson’s disease if in the dopaminergic neurons of the substantia nigra pars compacta,<sup>517</sup> or to more widespread cell disappearance is diseases such as those mediated by prions (e.g. ref. 7 and 518). Note of course that LPS is actually used to induce a form of Parkinson’s disease in experimental animals.<sup>4,230–237</sup>



**Fig. 11** Whole blood smears from a thrombo-embolic ischemic stroke patient. (A) Hyperactivated platelet mass with pseudopodia and microparticle formation. (B) Hyperactivated platelet mass showing fusion with spontaneously formed fibrin fibres, in whole blood. This activated mass is closely associated with an abnormally shaped erythrocyte covered with plasma proteins/microparticles, either from apoptotic hyperactivated platelets or damaged erythrocytes. Explanation of asterisks and arrows: in (A): red asterisk: platelet pseudopodia formation; blue arrows: microparticles. In (B): blue arrows: microparticles; green asterisk spontaneous fibrin fibre formation merging with pseudopodia formation from a hyperactivated, spread platelet (white asterisk). Note significant pseudopodia/fibrin extending from the spread platelet mass (white asterisk). Bacterium (pseudo-coloured in yellow-brown) is shown with a white block drawn around it.



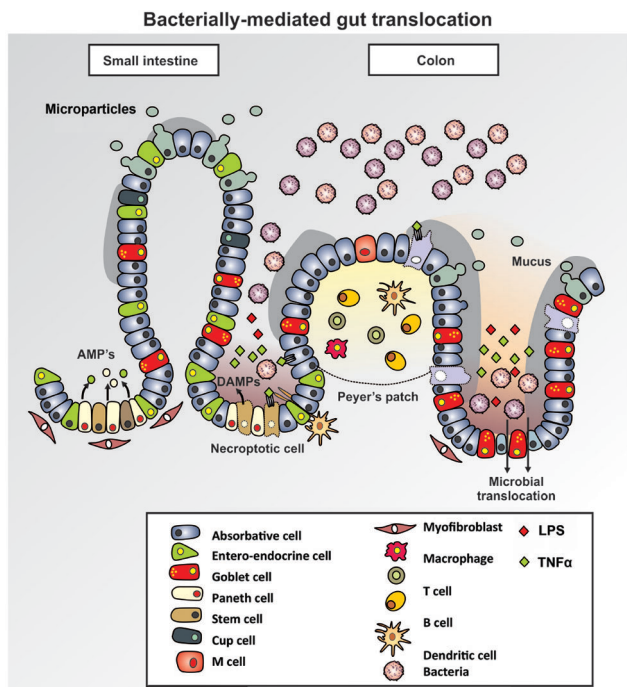


Fig. 12 Some of the mechanisms of bacterial translocation from the intestine in a 'leaky gut', based on ref. 430. Some cells (and LPS) may pass through tight junctions, while others pass through Peyer's patches or through spaces vacated by dead cells whose death may be initiated by LPS. Diagram is not to scale.

Within the present framework, there are at least three general and straightforward explanations for this differentiation of diseases despite a broad common cause. The first is that (as with infectious diseases), the nature of the microorganisms differs, with some of these inflammatory diseases clearly being more associated with some microbes than are others (*e.g.* ref. 5, 43 and 64). The second is simply that the tissue location of the dormant bacteria differs. The third follows as much from our ignorance of the molecular details, as discussed above, as from what we know – namely the fact that we know little of the different effects of LPS type and concentration, and the effects of different levels of cytokines and other molecules that may themselves vary this. Sharpening these kinds of analyses will obviously feature as an important area for future research, and we rehearse the first two points briefly.

### Differences in the organisms forming the dormant blood microbiome

We<sup>5</sup> and others (*e.g.* ref. 36, 43, 48, 49, 52, 62, 64 and 519–522) have provided both (ultra)microscopic and molecular (sequence-based) evidence for a very great variety of culture-negative organisms that have been noted to be present in the blood, despite the fact that it is normally considered to be sterile.

### Differences in the tissue locations of dormant bacteria

In a similar vein, microbes – presumably persistent, dormant organisms – are regularly detected in other tissues in which any

degree of proliferation would be highly inimical to the host. Some recent examples involving just reproductive disorders include the vagina,<sup>523–525</sup> the placenta,<sup>526–530</sup> and the amniotic fluid,<sup>531–540</sup> while recent evidence has also been provided for a sub-epidermal microbiome.<sup>541</sup> The considerable evidence for a dormant CNS microbiome was discussed earlier, and we here note the presence in the brain of TLR4 and its major role in neurodegeneration,<sup>542</sup> consistent with the idea that waking up dormant microbes can stimulate overt neurodegenerative disease.

## A note on autoantibodies

A number of the diseases (*e.g.* rheumatoid arthritis, multiple sclerosis, psoriasis) for which we are invoking a microbial component involving LPS are usually considered to be auto-immune diseases. The question then arises as to the origins of this autoimmunity. If LPS were a protein with a defined structure it would be relatively easy to compare its epitope sequences with those of the targets of host antibodies in different circumstances (*e.g.* ref. 543–546), but of course it is not. This said, there is plenty of evidence that host autoantibodies are elicited by LPS that have less than perfect specificity for the immunogen (*e.g.* ref. 547–558), so while most of this work is not very recent, this question of LPS-induced antibody non-specificity seems an avenue well worth exploring. We note too the potential toxicity of exogenously administered anti-LPS antibodies.<sup>559,560</sup> Regarding an autoimmune hypothesis, Marshall, Proal and colleagues highlight precisely this, along with a role for the vitamin D receptor.<sup>35,36,561–565</sup>

## Other hallmarks, and the role of iron dysregulation

While LPS itself as commonly measured seems to be a rather inadequate biomarker for chronic, inflammatory disease (Table 2), LBP (Table 1) and longer-lived markers of LPS exposure like IgM and IgA antibodies<sup>4,543–545,566–570</sup> may be more promising.

What serves to wake up the dormant microbes is not yet clear, and it is unlikely to be a single element. One possibility is certainly host stress as reflected in noradrenaline levels, a mechanism championed with considerable evidence (at least in terms of stimulating the growth of Gram-negative organisms) by Lyte, Freestone and colleagues.<sup>215,222,571–579</sup> We note that catecholamine synthesis may be induced in macrophages by LPS,<sup>580,581</sup> and that catecholamines increase inflammation<sup>582,583</sup> (another positive feedback loop). It is of special interest here that noradrenaline can act as an iron chelator,<sup>584–586</sup> since iron is normally seen as the nutrient most limiting to bacterial growth *in vivo* (*e.g.* ref. 16, 19–21 and 587–591). As we have pointed out before,<sup>6–10</sup> the diseases highlighted here are precisely those where iron dysregulation, inflammation, coagulopathies, microparticles,<sup>592,593</sup> and now a microbial component involving LPS *sensu lato*, are seen to coexist.



## Concluding remarks, areas of ignorance, and therapeutic potential

It is undoubtedly the case that despite our greatly improved understanding of many of the pathways involved in inflammation and sepsis (*sensu lato*), strategies for drug development have been noted mainly by their relative failure,<sup>411,594–597</sup> probably in significant measure because they aim at individual targets (see ref. 297). As a systems problem, the way to improve our therapeutic strategies is to recognise that there are multiple targets or steps. We have here stressed that LPS (*sensu lato*) created by the resuscitation of dormant bacteria is likely heavily involved, and that the latter can be affected greatly by the concentrations of free iron (that may also be a target for therapy). Fig. 13 shows some of the various translocations of dormant and proliferating bacteria between the different tissues, and the various steps that might thus be targeted. Improving methods for quantitative lipidomics (e.g. ref. 598–603) are likely to be of considerable value here.

Here we have stressed the significance of LPS, but initially promising trials of an anti-LPS antibody (HA-1A, Centoxin)<sup>604</sup> were not sustained (as is common<sup>605,606</sup>) because of ineffectiveness<sup>607,608</sup> or toxicity.<sup>559,560</sup> This was probably because of its lack of specificity between lipid A and other hydrophobic ligands,<sup>609</sup> and drug promiscuity is a function of hydrophobicity more generally (e.g. ref. 173, 468 and 610–612). Recognising that factor C, the active component of the *Limulus* amoebocyte lysate,<sup>94</sup> necessarily binds to LPS, Ding, Wohland and colleagues have isolated so-called sushi peptides therefrom,<sup>613–620</sup> in particular

sushi peptides S1 and S3. These are 34mers that that can bind to and inactivate endotoxin molecules (and are in some cases directly antibacterial), in concert with bacterial phospholipids.<sup>621</sup> They are said to have low human cell toxicity, and these peptides (or variants<sup>622,623</sup>) would seem to have significant therapeutic potential. As a hydrophobic target, variant lipocalins binding LPS ('anticalins'<sup>624–628</sup>) might also be of value here. The evidence that HDL can bind to and sequester LPS was given above.

It is hard to gauge whether a better treatment than an anti-LPS antibody might involve recombinant LBP because while LBP can sequester LPS it can also transfer it to TLR4.<sup>127,134,629–631</sup> Probably the best strategy is to avoid letting the bacteria proliferate in an uncontrolled manner at all, and to this end an iron-withholding strategy<sup>632</sup> seems most suitable (e.g. ref. 6, 7 and 633–636) (and not a fortification one<sup>637,638</sup>). In a similar vein, many of the substances found to be anti-inflammatory are so because of their iron-chelating properties (e.g. ref. 7 and 639–641), though clearly there are a vast number of possible steps (plural) at which anti-inflammatory substances may act. Those involving downstream cytokines (e.g. ref. 539) are outwith our scope here.

In addition, provided one can avoid excessive LPS shedding and a Jarisch–Herxheimer reaction,<sup>266,268</sup> suitable antibiotics (e.g. minocycline<sup>642–650</sup>) that have polypharmacological properties<sup>651,652</sup> must certainly have a role in treating purportedly non-communicable inflammatory diseases, as well as those established as infectious. It is at least worthy of mention,<sup>653</sup> albeit drugs can be quite promiscuous with regard to hitting multiple targets,<sup>173,466,651,652,654</sup> that certain antibiotics are both used and effective in the treatment of diseases such as multiple sclerosis,<sup>655–661</sup> rheumatoid arthritis,<sup>544,642,651,652,662–665</sup> and psoriasis,<sup>666–668</sup> while vaccination can also work to protect against supposedly non-communicable disease.<sup>669,670</sup>

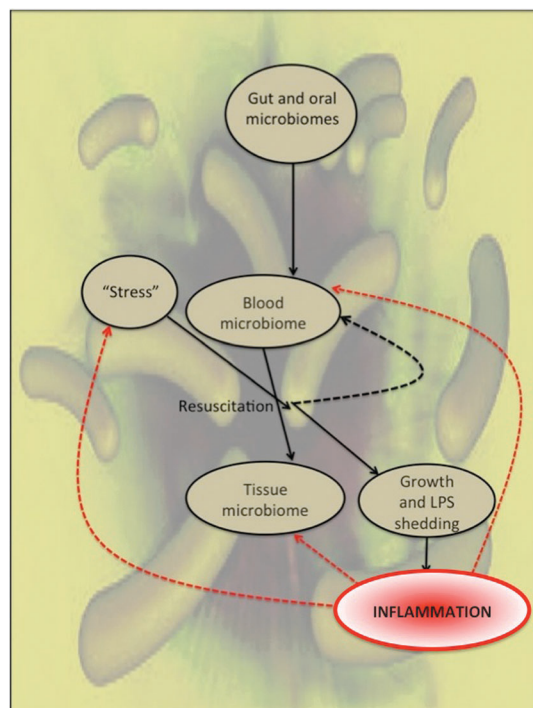
A modern trend is to use patients as their own controls (so-called  $n = 1$ <sup>671</sup> or  $n$ -of-1<sup>672,673</sup> methods), in suitable cross-over designs. These seem ideally suited to chronic diseases of the type discussed here, especially for those in which disease severity can itself change significantly ('flare'<sup>674–677</sup>) on quite rapid timescales (for the large and very numerous circadian changes in a mammalian transcriptome, see ref. 678). Indeed, we predict that such flares will be accompanied by major changes in the relevant microbiomes. But to understand the precise mechanisms involved we shall need much better, more quantitative, and above all reliable methods for measuring and manipulating the microbes, pathways and pathway elements involved. This is the most urgent task for the future.

## Note added in proof

A recent and complementary article highlights the role of LPS translocation directly from the gut.<sup>679</sup>

## Acknowledgements

We thank Dr David Brough for commenting on the manuscript and Prof Ian Roberts for drawing our attention to the literature



**Fig. 13** Some of the various translocations of dormant and proliferating bacteria between different tissues as discussed herein. The red dotted lines indicate the influence of inflammation, while the resuscitation step is indicated by the black dotted line. The solid black lines likely involve some kind of translocation.



on quiescent intracellular reservoirs. We thank the Biotechnology and Biological Sciences Research Council (grant BB/L025752/1) as well as the National Research Foundation (NRF) of South Africa for supporting this collaboration. This is also a contribution from the Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals (SYNBIOCHEM) (BBSRC grant BB/M017702/1).

## References

- Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, ed. J. E. Bennett, R. Dolin and M. J. Blaser, Saunders Elsevier, Philadelphia, 8th edn, 2015.
- A. S. Kaprelyants, J. C. Gottschal and D. B. Kell, Dormancy in non-sporulating bacteria, *FEMS Microbiol. Rev.*, 1993, **10**, 271–286.
- D. B. Kell, A. S. Kaprelyants, D. H. Weichart, C. L. Harwood and M. R. Barer, Viability and activity in readily culturable bacteria: a review and discussion of the practical issues, *Antonie van Leeuwenhoek*, 1998, **73**, 169–187.
- D. B. Kell, M. Potgieter and E. Pretorius, Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology, *F1000Research*, 2015, **4**, 179.
- M. Potgieter, J. Bester, D. B. Kell and E. Pretorius, The dormant blood microbiome in chronic, inflammatory diseases, *FEMS Microbiol. Rev.*, 2015, **39**, 567–591.
- D. B. Kell, Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases, *BMC Med. Genomics*, 2009, **2**, 2.
- D. B. Kell, Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples, *Arch. Toxicol.*, 2010, **577**, 825–889.
- D. B. Kell and E. Pretorius, Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells, *Metallomics*, 2014, **6**, 748–773.
- D. B. Kell and E. Pretorius, The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen), *Integr. Biol.*, 2015, **7**, 24–52.
- E. Pretorius and D. B. Kell, Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases, *Integr. Biol.*, 2014, **6**, 486–510.
- R. L. Jurado, Iron, infections, and anemia of inflammation, *Clin. Infect. Dis.*, 1997, **25**, 888–895.
- M. A. Fischbach, H. N. Lin, D. R. Liu and C. T. Walsh, How pathogenic bacteria evade mammalian sabotage in the battle for iron, *Nat. Chem. Biol.*, 2006, **2**, 132–138.
- S. T. Ong, J. Z. S. Ho, B. Ho and J. L. Ding, Iron-withholding strategy in innate immunity, *Immunobiology*, 2006, **211**, 295–314.
- M. Miethke and M. A. Marahiel, Siderophore-based iron acquisition and pathogen control, *Microbiol. Mol. Biol. Rev.*, 2007, **71**, 413–451.
- L. J. Wang and B. J. Cherayil, Ironing out the wrinkles in host defense: interactions between iron homeostasis and innate immunity, *J. Innate Immun.*, 2009, **1**, 455–464.
- B. C. Chu, A. Garcia-Herrero, T. H. Johanson, K. D. Krewulak, C. K. Lau, R. S. Peacock, Z. Slavinskaya and H. J. Vogel, Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view, *BioMetals*, 2010, **23**, 601–611.
- K. E. Sivick and H. L. T. Mobley, Waging war against uropathogenic *Escherichia coli*: winning back the urinary tract, *Infect. Immun.*, 2010, **78**, 568–585.
- M. Wessling-Resnick, Iron homeostasis and the inflammatory response, *Annu. Rev. Nutr.*, 2010, **30**, 105–122.
- K. P. Haley and E. P. Skaar, A battle for iron: host sequestration and *Staphylococcus aureus* acquisition, *Microbes Infect.*, 2012, **14**, 217–227.
- A. E. Armitage and H. Drakesmith, The battle for iron, *Science*, 2014, **346**, 1299–1300.
- S. Subashchandrabose and H. L. T. Mobley, Back to the metal age: battle for metals at the host–pathogen interface during urinary tract infection, *Metallomics*, 2015, **7**, 935–942.
- L. Diacovich and J. P. Gorvel, Bacterial manipulation of innate immunity to promote infection, *Nat. Rev. Microbiol.*, 2010, **8**, 117–128.
- A. Plóciennikowska, A. Hromada-Judycka, K. Borzcka and K. Kwiatkowska, Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling, *Cell. Mol. Life Sci.*, 2015, **72**, 557–581.
- S. Morath, A. Geyer and T. Hartung, Structure–function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*, *J. Exp. Med.*, 2001, **193**, 393–397.
- B. Luef, K. R. Frischkorn, K. C. Wrighton, H. Y. Holman, G. Birarda, B. C. Thomas, A. Singh, K. H. Williams, C. E. Siegerist, S. G. Tringe, K. H. Downing, L. R. Comolli and J. F. Banfield, Diverse uncultivated ultra-small bacterial cells in groundwater, *Nat. Commun.*, 2015, **6**, 6372.
- P. B. Eckburg, P. W. Lepp and D. A. Relman, Archaea and their potential role in human disease, *Infect. Immun.*, 2003, **71**, 591–596.
- P. Blais Lecours, C. Duchaine, M. Taillefer, C. Tremblay, M. Veillette, Y. Cormier and D. Marsolais, Immunogenic properties of archaeal species found in bioaerosols, *PLoS One*, 2011, **6**, e23326.
- B. Dridi, Laboratory tools for detection of archaea in humans, *Clin. Microbiol. Infect.*, 2012, **18**, 825–833.
- R. I. Aminov, Role of archaea in human disease, *Front. Cell. Infect. Microbiol.*, 2013, **3**, 42.
- R. F. Itzhaki, Herpes simplex virus type 1 and Alzheimer's disease: increasing evidence for a major role of the virus, *Front. Aging Neurosci.*, 2014, **6**, 202.
- C. H. Chiang, C. C. Huang, W. L. Chan, Y. C. Chen, T. J. Chen, S. J. Lin, J. W. Chen and H. B. Leu, Association between *Mycoplasma pneumonia* and increased risk of





- ischemic stroke: a nationwide study, *Stroke*, 2011, **42**, 2940–2943.
- 32 C. Gil, A. A. S. González, I. L. León, A. Rivera, R. S. Olea and L. Cedillo, Detection of Mycoplasmas in Patients with Amyotrophic Lateral Sclerosis, *Adv. Microbiol.*, 2014, **4**, 712–719.
- 33 G. L. Nicolson, R. Gan, N. L. Nicolson and J. Haier, Evidence for mycoplasma ssp., *Chlamydia pneumoniae*, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders, *J. Neurosci. Res.*, 2007, **85**, 1143–1148.
- 34 T. Buzan, *How to mind map*, Thorsons, London, 2002.
- 35 A. D. Proal, P. J. Albert and T. G. Marshall, The human microbiome and autoimmunity, *Curr. Opin. Rheumatol.*, 2013, **25**, 234–240.
- 36 A. D. Proal, P. J. Albert and T. G. Marshall, Inflammatory disease and the human microbiome, *Discov. Med.*, 2014, **17**, 257–265.
- 37 D. B. Kell, A. S. Kaprelyants and A. Grafen, On pheromones, social behaviour and the functions of secondary metabolism in bacteria, *Trends Ecol. Evolution*, 1995, **10**, 126–129.
- 38 G. V. Mukamolova, A. S. Kaprelyants, D. B. Kell and M. Young, Adoption of the transiently non-culturable state – a bacterial survival strategy?, *Adv. Micr. Physiol.*, 2003, **47**, 65–129.
- 39 S. V. Avery, Microbial cell individuality and the underlying sources of heterogeneity, *Nat. Rev. Microbiol.*, 2006, **4**, 577–587.
- 40 N. Q. Balaban, Persistence: mechanisms for triggering and enhancing phenotypic variability, *Curr. Opin. Genet. Dev.*, 2011, **21**, 768–775.
- 41 S. S. Epstein, The phenomenon of microbial uncultivability, *Curr. Opin. Microbiol.*, 2013, **16**, 636–642.
- 42 P. W. Ewald, *Evolution of infectious disease*, Oxford University Press, New York, 1994.
- 43 P. W. Ewald, *Plague time: the new germ theory of disease*, Anchor Books, New York, 2002.
- 44 P. W. Ewald, Evolution of virulence, *Infectious disease clinics of North America*, 2004, **18**, 1–15.
- 45 M. T. Green, P. M. Heidger Jr. and G. Domingue, Proposed reproductive cycle for a relatively stable L-phase variant of *Streptococcus faecalis*, *Infect. Immun.*, 1974, **10**, 915–927.
- 46 M. T. Green, P. M. Heidger Jr. and G. Domingue, Demonstration of the phenomena of microbial persistence and reversion with bacterial L-forms in human embryonic kidney cells, *Infect. Immun.*, 1974, **10**, 889–914.
- 47 G. J. Domingue and J. U. Schlegel, Novel bacterial structures in human blood: cultural isolation, *Infect. Immun.*, 1977, **15**, 621–627.
- 48 S. Nikkari, I. J. McLaughlin, W. Bi, D. E. Dodge and D. A. Relman, Does blood of healthy subjects contain bacterial ribosomal DNA?, *J. Clin. Microbiol.*, 2001, **39**, 1956–1959.
- 49 K. Moriyama, C. Ando, K. Tashiro, S. Kuhara, S. Okamura, S. Nakano, Y. Takagi, T. Miki, Y. Nakashima and H. Hirakawa, Polymerase chain reaction detection of bacterial 16S rRNA gene in human blood, *Microbiol. Immunol.*, 2008, **52**, 375–382.
- 50 A. Cherkaoui, S. Emonet, D. Ceroni, B. Candolfi, J. Hibbs, P. Francois and J. Schrenzel, Development and validation of a modified broad-range 16S rDNA PCR for diagnostic purposes in clinical microbiology, *J. Microbiol. Methods*, 2009, **79**, 227–231.
- 51 S. G. Sakka, A. J. Kochem, C. Disqué and N. Wellinghausen, Blood infection diagnosis by 16S rDNA broad-spectrum polymerase chain reaction: the relationship between antibiotic treatment and bacterial DNA load, *Anesth. Analg.*, 2009, **109**, 1707–1708.
- 52 J. Amar, M. Serino, C. Lange, C. Chabo, J. Iacovoni, S. Mondot, P. Lepage, C. Klopp, J. Mariette, O. Bouchez, L. Perez, M. Courtney, M. Marre, P. Klopp, O. Lantieri, J. Doré, M. A. Charles, B. Balkau, R. Burcelin and D. S. Grp, Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept, *Diabetologia*, 2011, **54**, 3055–3061.
- 53 K. Grif, I. Heller, W. M. Prodingner, K. Lechleitner, C. Lass-Flörl and D. Orth, Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis, *J. Clin. Microbiol.*, 2012, **50**, 2250–2254.
- 54 K. Grif, M. Fille, R. Wurzner, G. Weiss, I. Lorenz, G. Gruber, S. Eschertzhuber, D. Nachbaur, C. Lass-Flörl and D. Orth, Rapid detection of bloodstream pathogens by real-time PCR in patients with sepsis, *Wien. Klin. Wochenschr.*, 2012, **124**, 266–270.
- 55 A. Fernández-Cruz, M. Marin, M. Kestler, L. Alcalá, M. Rodríguez-Crèixems and E. Bouza, The value of combining blood culture and SeptiFast data for predicting complicated bloodstream infections caused by Gram-positive bacteria or *Candida* species, *J. Clin. Microbiol.*, 2013, **51**, 1130–1136.
- 56 P. Gaibani, M. Mariconti, G. Bua, S. Bonora, D. Sasserà, M. P. Landini, P. Mulatto, S. Novati, C. Bandi and V. Sambri, Development of a broad-range 23S rDNA real-time PCR assay for the detection and quantification of pathogenic bacteria in human whole blood and plasma specimens, *BioMed Res. Int.*, 2013, **2013**, 264651.
- 57 J. Sato, A. Kanazawa, F. Ikeda, T. Yoshihara, H. Goto, H. Abe, K. Komiya, M. Kawaguchi, T. Shimizu, T. Ogihara, Y. Tamura, Y. Sakurai, R. Yamamoto, T. Mita, Y. Fujitani, H. Fukuda, K. Nomoto, T. Takahashi, T. Asahara, T. Hirose, S. Nagata, Y. Yamashiro and H. Watada, Gut dysbiosis and detection of “live gut bacteria” in blood of Japanese patients with type 2 diabetes, *Diabetes Care*, 2014, **37**, 2343–2350.
- 58 C. L. Liu, H. W. Ai, W. P. Wang, L. Chen, H. B. Hu, T. Ye, X. H. Zhu, F. Wang, Y. L. Liao, Y. Wang, G. Ou, L. Xu, M. Sun, C. Jian, Z. J. Chen, L. Li, B. Zhang, L. Tian, B. Wang, S. Yan and Z. Y. Sun, Comparison of 16S rRNA gene PCR and blood culture for diagnosis of neonatal sepsis, *Arch. Pediatr.*, 2014, **21**, 162–169.



- 59 F. Valencia-Shelton and M. Loeffelholz, Nonculture techniques for the detection of bacteremia and fungemia, *Future Microbiol.*, 2014, **9**, 543–559.
- 60 G. Domingue, B. Turner and J. U. Schlegel, Cell-wall deficient bacterial variants in kidney tissue. Detection by immunofluorescence, *Urology*, 1974, **3**, 288–292.
- 61 G. J. Domingue, Electron dense cytoplasmic particles and chronic infection – a bacterial pleomorphism hypothesis, *Endocytobiosis Cell Res.*, 1995, **11**, 19–40.
- 62 G. J. Domingue and H. B. Woody, Bacterial persistence and expression of disease, *Clin. Microbiol. Rev.*, 1997, **10**, 320–344.
- 63 G. J. Domingue, Demystifying pleomorphic forms in persistence and expression of disease: are they bacteria and is peptidoglycan the solution?, *Discov. Med.*, 2010, **10**, 234–246.
- 64 L. Mattman, *Cell wall deficient forms: stealth pathogens*, CRC Press, Boca Raton, 3rd edn, 2001.
- 65 M. Miskinyte, A. Sousa, R. S. Ramiro, J. A. de Sousa, J. Kotlinowski, I. Caramalho, S. Magalhães, M. P. Soares and I. Gordo, The genetic basis of *Escherichia coli* pathoadaptation to macrophages, *PLoS Pathog.*, 2013, **9**, e1003802.
- 66 M. Miskinyte and I. Gordo, Increased survival of antibiotic-resistant *Escherichia coli* inside macrophages, *Antimicrob. Agents Chemother.*, 2013, **57**, 189–195.
- 67 P. Liehl, V. Zuzarte-Luis and M. M. Mota, Unveiling the pathogen behind the vacuole, *Nat. Rev. Microbiol.*, 2015, **13**, 589–598.
- 68 D. Ribet and P. Cossart, How bacterial pathogens colonize their hosts and invade deeper tissues, *Microbes Infect.*, 2015, **17**, 173–183.
- 69 G. E. Thwaites and V. Gant, Are bloodstream leukocytes Trojan Horses for the metastasis of *Staphylococcus aureus*?, *Nat. Rev. Microbiol.*, 2011, **9**, 215–222.
- 70 F. C. O. Los, T. M. Randis, R. V. Aroian and A. J. Ratner, Role of pore-forming toxins in bacterial infectious diseases, *Microbiol. Mol. Biol. Rev.*, 2013, **77**, 173–207.
- 71 T. J. LaRocca, E. A. Stivison, E. A. Hod, S. L. Spitalnik, P. J. Cowan, T. M. Randis and A. J. Ratner, Human-specific bacterial pore-forming toxins induce programmed necrosis in erythrocytes, *mBio*, 2014, **5**, e01251–01214.
- 72 A. K. May, T. G. Gleason, R. G. Sawyer and T. L. Pruett, Contribution of *Escherichia coli* alpha-hemolysin to bacterial virulence and to intraperitoneal alterations in peritonitis, *Infect. Immun.*, 2000, **68**, 176–183.
- 73 L. Ferrier, L. Mazelin, N. Cenac, P. Desreumaux, A. Janin, D. Emilie, J. F. Colombel, R. Garcia-Villar, J. Fioramonti and L. Bueno, Stress-induced disruption of colonic epithelial barrier: role of interferon-gamma and myosin light chain kinase in mice, *Gastroenterology*, 2003, **125**, 795–804.
- 74 K. Honda and D. R. Littman, The microbiome in infectious disease and inflammation, *Annu. Rev. Immunol.*, 2012, **30**, 759–795.
- 75 N. R. Klatt, N. T. Funderburg and J. M. Brenchley, Microbial translocation, immune activation, and HIV disease, *Trends Microbiol.*, 2013, **21**, 6–13.
- 76 P. J. Turnbaugh, R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight and J. I. Gordon, The human microbiome project, *Nature*, 2007, **449**, 804–810.
- 77 W. S. Garrett, Cancer and the microbiota, *Science*, 2015, **348**, 80–86.
- 78 J. R. Caso, O. Hurtado, M. P. Pereira, B. Garcia-Bueno, L. Menchen, L. Alou, M. L. Gomez-Lus, M. A. Moro, I. Lizasoain and J. C. Leza, Colonic bacterial translocation as a possible factor in stress-worsening experimental stroke outcome, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2009, **296**, R979–R985.
- 79 R. R. Schumann and J. Zweigner, A novel acute-phase marker: lipopolysaccharide binding protein (LBP), *Clin. Chem. Lab. Med.*, 1999, **37**, 271–274.
- 80 R. L. Kitchens and P. A. Thompson, Impact of sepsis-induced changes in plasma on LPS interactions with monocytes and plasma lipoproteins: roles of soluble CD14, LBP, and acute phase lipoproteins, *J. Endotoxin Res.*, 2003, **9**, 113–118.
- 81 R. R. Schumann, Old and new findings on lipopolysaccharide-binding protein: a soluble pattern-recognition molecule, *Biochem. Soc. Trans.*, 2011, **39**, 989–993.
- 82 P. H. Ding and L. J. Jin, The role of lipopolysaccharide-binding protein in innate immunity: a revisit and its relevance to oral/periodontal health, *J. Periodontal Res.*, 2014, **49**, 1–9.
- 83 A. Gonzalez-Quintela, M. Alonso, J. Campos, L. Vizcaino, L. Loidi and F. Gude, Determinants of serum concentrations of Lipopolysaccharide-Binding Protein (LBP) in the adult population: the role of obesity, *PLoS One*, 2013, **8**, e54600.
- 84 J. Zweigner, R. R. Schumann and J. R. Weber, The role of lipopolysaccharide-binding protein in modulating the innate immune response, *Microbes Infect.*, 2006, **8**, 946–952.
- 85 M. Van Oosten, P. C. Rensen, E. S. Van Amersfoort, M. Van Eck, A. M. Van Dam, J. J. Breve, T. Vogel, A. Panet, T. J. Van Berkel and J. Kuiper, Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality. A new therapeutic approach to treat Gram-negative sepsis, *J. Biol. Chem.*, 2001, **276**, 8820–8824.
- 86 C. Cuaz-Pérolin, L. Billiet, E. Baugé, C. Copin, D. Scott-Algara, F. Genze, B. Buchele, T. Syrovets, T. Simmet and M. Rouis, Antiinflammatory and antiatherogenic effects of the NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged ApoE<sup>-/-</sup> mice, *Arterioscler., Thromb., Vasc. Biol.*, 2008, **28**, 272–277.
- 87 N. Urosevic and R. N. Martins, Infection and Alzheimer's disease: the APOE epsilon4 connection and lipid metabolism, *J. Alzheimer's Dis.*, 2008, **13**, 421–435.
- 88 R. F. Itzhaki and M. A. Wozniak, Herpes simplex virus type 1, apolipoprotein E, and cholesterol: a dangerous liaison in Alzheimer's disease and other disorders, *Prog. Lipid Res.*, 2006, **45**, 73–90.
- 89 V. Leoni, The effect of apolipoprotein E (ApoE) genotype on biomarkers of amyloidogenesis, tau pathology and neurodegeneration in Alzheimer's disease, *Clin. Chem. Lab. Med.*, 2011, **49**, 375–383.



- 90 C. C. Liu, T. Kanekiyo, H. Xu and G. Bu, Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy, *Nat. Rev. Neurol.*, 2013, **9**, 106–118.
- 91 E. Dorey, N. Chang, Q. Y. Liu, Z. Yang and W. Zhang, Apolipoprotein E, amyloid-beta, and neuroinflammation in Alzheimer's disease, *Neurosci. Bull.*, 2014, **30**, 317–330.
- 92 L. M. Tai, S. Mehra, V. Shete, S. Estus, G. W. Rebeck, G. Bu and M. J. LaDu, Soluble apoE/Abeta complex: mechanism and therapeutic target for APOE4-induced AD risk, *Mol. Neurodegener.*, 2014, **9**, 2.
- 93 G. S. Zaman and F. Zaman, Relationship between postprandial endotoxemia in nonobese postmenopausal women and diabetic nonobese postmenopausal women, *J. Nat. Sci., Biol. Med.*, 2015, **6**, 89–93.
- 94 J. L. Ding and B. Ho, Endotoxin detection—from limulus amoebocyte lysate to recombinant factor C, *Subcell. Biochem.*, 2010, **53**, 187–208.
- 95 M. Lan, J. Wu, W. Liu, W. Zhang, J. Ge, H. Zhang, J. Sun, W. Zhao and P. Wang, Copolythiophene-derived colorimetric and fluorometric sensor for visually supersensitive determination of lipopolysaccharide, *J. Am. Chem. Soc.*, 2012, **134**, 6685–6694.
- 96 W. Su, S. E. Kim, M. Cho, J. D. Nam, W. S. Choe and Y. Lee, Selective detection of endotoxin using an impedance aptasensor with electrochemically deposited gold nanoparticles, *Innate Immun.*, 2013, **19**, 388–397.
- 97 A. P. Das, P. S. Kumar and S. Swain, Recent advances in biosensor based endotoxin detection, *Biosens. Bioelectron.*, 2014, **51**, 62–75.
- 98 R. L. Kitchens, G. Wolfbauer, J. J. Albers and R. S. Munford, Plasma lipoproteins promote the release of bacterial lipopolysaccharide from the monocyte cell surface, *J. Biol. Chem.*, 1999, **274**, 34116–34122.
- 99 W. Khovidhunkit, M. S. Kim, R. A. Memon, J. K. Shigenaga, A. H. Moser, K. R. Feingold and C. Grunfeld, Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host, *J. Lipid Res.*, 2004, **45**, 1169–1196.
- 100 J. H. M. Levels, P. R. Abraham, E. P. van Barneveld, J. C. M. Meijers and S. J. G. van Deventer, Distribution and kinetics of lipoprotein-bound lipoteichoic acid, *Infect. Immun.*, 2003, **71**, 3280–3284.
- 101 P. Fraunberger, S. Schaefer, K. Werdan, A. K. Walli and D. Seidel, Reduction of circulating cholesterol and apolipoprotein levels during sepsis, *Clin. Chem. Lab. Med.*, 1999, **37**, 357–362.
- 102 J. H. M. Levels, P. Geurts, H. Karlsson, R. Marée, S. Ljunggren, L. Fornander, L. Wehenkel, M. Lindahl, E. S. G. Stroes, J. A. Kuivenhoven and J. C. M. Meijers, High-density lipoprotein proteome dynamics in human endotoxemia, *Proteome Sci.*, 2011, **9**, 34.
- 103 R. F. Wilson, J. F. Barletta and J. G. Tyburski, Hypocholesterolemia in sepsis and critically ill or injured patients, *Crit. Care*, 2003, **7**, 413–414.
- 104 J. Y. Chien, J. S. Jerng, C. J. Yu and P. C. Yang, Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis, *Crit. Care Med.*, 2005, **33**, 1688–1693.
- 105 A. L. Catapano, A. Pirillo, F. Bonacina and G. D. Norata, HDL in innate and adaptive immunity, *Cardiovasc. Res.*, 2014, **103**, 372–383.
- 106 L. Guo, J. Ai, Z. Zheng, D. A. Howatt, A. Daugherty, B. Huang and X. A. Li, High density lipoprotein protects against polymicrobe-induced sepsis in mice, *J. Biol. Chem.*, 2013, **288**, 17947–17953.
- 107 A. Pirillo, A. L. Catapano and G. D. Norata, HDL in infectious diseases and sepsis, *Handb. Exp. Pharmacol.*, 2015, **224**, 483–508.
- 108 G. D. Hitchens and D. B. Kell, Uncouplers can shuttle rapidly between localised energy coupling sites during photophosphorylation by chromatophores of *Rhodospseudomonas capsulata* N22, *Biochem. J.*, 1983, **212**, 25–30.
- 109 G. D. Hitchens and D. B. Kell, On the functional unit of energy coupling in photophosphorylation by bacterial chromatophores, *Biochim. Biophys. Acta*, 1983, **723**, 308–316.
- 110 D. B. Kell, H. M. Ryder, A. S. Kaprelyants and H. V. Westerhoff, Quantifying heterogeneity: flow cytometry of bacterial cultures, *Antonie van Leeuwenhoek*, 1991, **60**, 145–158.
- 111 H. M. Davey and D. B. Kell, Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analysis, *Microbiol. Rev.*, 1996, **60**, 641–696.
- 112 S. Zielen, J. Trischler and R. Schubert, Lipopolysaccharide challenge: immunological effects and safety in humans, *Expert Rev. Clin. Immunol.*, 2015, **11**, 409–418.
- 113 C. M. Harris and D. B. Kell, The estimation of microbial biomass, *Biosensors*, 1985, **1**, 17–84.
- 114 S. W. Watson, T. J. Novitsky, H. L. Quinby and F. W. Valois, Determination of bacterial number and biomass in the marine environment, *Appl. Environ. Microbiol.*, 1977, **33**, 940–946.
- 115 F. C. Pearson, M. E. Weary, H. E. Sargent, T. J. Novitsky, H. Lin, G. Lindsay, R. N. Berzofsky, A. L. Lane, J. D. Wilson, J. F. Cooper, E. J. Helme, C. W. Twohy, H. I. Basch, M. A. Rech, J. W. Slade and M. P. Winegar, Comparison of several control standard endotoxins to the National Reference Standard Endotoxin—an HIMA collaborative study, *Appl. Environ. Microbiol.*, 1985, **50**, 91–93.
- 116 J. Andrä, P. Garidel, A. Majerle, R. Jerala, R. Ridge, E. Paus, T. Novitsky, M. H. Koch and K. Brandenburg, Biophysical characterization of the interaction of *Limulus polyphemus* endotoxin neutralizing protein with lipopolysaccharide, *Eur. J. Biochem.*, 2004, **271**, 2037–2046.
- 117 P. A. Ketchum and T. J. Novitsky, Assay of endotoxin by *Limulus* amoebocyte lysate, *Methods Mol. Med.*, 2000, **36**, 3–12.
- 118 I. Mattsby-Baltzer, K. Lindgren, B. Lindholm and L. Edebo, Endotoxin shedding by enterobacteria: free and cell-bound endotoxin differ in *Limulus* activity, *Infect. Immun.*, 1991, **59**, 689–695.
- 119 T. J. Novitsky, Limitations of the *Limulus* amoebocyte lysate test in demonstrating circulating lipopolysaccharides, *Ann. N. Y. Acad. Sci.*, 1998, **851**, 416–421.



- 120 T. J. Novitsky, *Biomedical Applications of Limulus Amebocyte Lysate*, Biology and Conservation of Horseshoe Crabs, 2009, pp. 315–329.
- 121 A. Albillos, A. de la Hera, M. González, J. L. Moya, J. L. Calleja, J. Monserrat, L. Ruiz-del-Arbol and M. Alvarez-Mon, Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement, *Hepatology*, 2003, **37**, 208–217.
- 122 C. Elsing, S. Ernst, N. Kayali, W. Stremmel and S. Harenberg, Lipopolysaccharide binding protein, interleukin-6 and C-reactive protein in acute gastrointestinal infections: value as biomarkers to reduce unnecessary antibiotic therapy, *Infection*, 2011, **39**, 327–331.
- 123 N. T. Funderburg, S. R. Stubblefield Park, H. C. Sung, G. Hardy, B. Clagett, J. Ignatz-Hoover, C. V. Harding, P. Fu, J. A. Katz, M. M. Lederman and A. D. Levine, Circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells are activated in inflammatory bowel disease and are associated with plasma markers of inflammation, *Immunology*, 2013, **140**, 87–97.
- 124 H. Zhou, J. Hu, Q. Zhu, S. Yang, Y. Zhang, R. Gao, L. Liu, Y. Wang, Q. Zhen, Q. Lv and Q. Li, Lipopolysaccharide-binding protein cannot independently predict type 2 diabetes mellitus: a nested case-control study, *J. Diabetes*, 2015, DOI: 10.1111/1753-0407.12281.
- 125 T. Vollmer, C. Piper, K. Kleesiek and J. Dreier, Lipopolysaccharide-binding protein: a new biomarker for infectious endocarditis?, *Clin. Chem.*, 2009, **55**, 295–304.
- 126 L. Sun, Z. Yu, X. Ye, S. Zou, H. Li, D. Yu, H. Wu, Y. Chen, J. Dore, K. Clement, F. B. Hu and X. Lin, A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese, *Diabetes Care*, 2010, **33**, 1925–1932.
- 127 J. Zweigner, H. J. Gramm, O. C. Singer, K. Wegscheider and R. R. Schumann, High concentrations of lipopolysaccharide-binding protein in serum of patients with severe sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes, *Blood*, 2001, **98**, 3800–3808.
- 128 T. D. Sandahl, H. Gronbaek, H. J. Moller, S. Stoy, K. L. Thomsen, A. K. Dige, J. Agnholt, S. Hamilton-Dutoit, S. Thiel and H. Vilstrup, Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study, *Am. J. Gastroenterol.*, 2014, **109**, 1749–1756.
- 129 E. A. Tsalkidou, E. Roilides, S. Gardikis, G. Trypsianis, A. Kortsaris, A. Chatzimichael and I. Tentis, Lipopolysaccharide-binding protein: a potential marker of febrile urinary tract infection in childhood, *Pediatr. Nephrol.*, 2013, **28**, 1091–1097.
- 130 R. Berner, B. Füll, F. Stelter, J. Dröse, H. P. Müller and C. Schütt, Elevated levels of lipopolysaccharide-binding protein and soluble CD14 in plasma in neonatal early-onset sepsis, *Clin. Diagn. Lab. Immunol.*, 2002, **9**, 440–445.
- 131 J. L. Leante-Castellanos, L. G. de Gadiana-Romualdo, C. Fuentes-Gutiérrez, A. Hernando-Holgado, A. Garcia-González and E. Jiménez-Santos, The value of lipopolysaccharide binding protein for diagnosis of late-onset neonatal sepsis in very low birth weight infants, *J. Perinat. Med.*, 2015, **43**, 253–257.
- 132 A. H. M. Froom, M. A. Dentener, J. W. M. Greve, G. Ramsay and W. A. Buurman, Lipopolysaccharide toxicity-regulating proteins in bacteremia, *J. Infect. Dis.*, 1995, **171**, 1250–1257.
- 133 S. M. Opal, P. J. Scannon, J. L. Vincent, M. White, S. F. Carroll, J. E. Palardy, N. A. Parejo, J. P. Pribble and J. H. Lemke, Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock, *J. Infect. Dis.*, 1999, **180**, 1584–1589.
- 134 N. Lamping, R. Dettmer, N. W. Schröder, D. Pfeil, W. Hallatschek, R. Burger and R. R. Schumann, LPS-binding protein protects mice from septic shock caused by LPS or Gram-negative bacteria, *J. Clin. Invest.*, 1998, **101**, 2065–2071.
- 135 J. Villar, L. Pérez-Méndez, E. Espinosa, C. Flores, J. Blanco, A. Muriel, S. Basaldúa, M. Muros, L. Blanch, A. Artigas and R. M. Kacmarek, Grecia and Gen-Sep Groups, Serum lipopolysaccharide binding protein levels predict severity of lung injury and mortality in patients with severe sepsis, *PLoS One*, 2009, **4**, e6818.
- 136 M. Mierzchala, M. Krzystek-Korpacka, A. Gamian and G. Durek, Quantitative indices of dynamics in concentrations of lipopolysaccharide-binding protein (LBP) as prognostic factors in severe sepsis/septic shock patients – Comparison with CRP and procalcitonin, *Clin. Biochem.*, 2011, **44**, 357–363.
- 137 H. Ghanim, S. Abuaysheh, C. L. Sia, K. Korzeniewski, A. Chaudhuri, J. M. Fernandez-Real and P. Dandona, Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance, *Diabetes Care*, 2009, **32**, 2281–2287.
- 138 P. W. Hunt, J. Brenchley, E. Sinclair, J. M. McCune, M. Roland, K. Page-Shafer, P. Hsue, B. Emu, M. Krone, H. Lampiris, D. Douek, J. N. Martin and S. G. Deeks, Relationship between T cell activation and CD4<sup>+</sup> T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy, *J. Infect. Dis.*, 2008, **197**, 126–133.
- 139 A. L. Harte, N. F. da Silva, S. J. Creely, K. C. McGee, T. Billyard, E. M. Youssef-Elabd, G. Tripathi, E. Ashour, M. S. Abdalla, H. M. Sharada, A. I. Amin, A. D. Burt, S. Kumar, C. P. Day and P. G. McTernan, Elevated endotoxin levels in non-alcoholic fatty liver disease, *J. Inflammation*, 2010, **7**, 15.
- 140 J. C. Marshall, P. M. Walker, D. M. Foster, D. Harris, M. Ribeiro, J. Paice, A. D. Romaschin and A. N. Derzko, Measurement of endotoxin activity in critically ill patients using whole blood neutrophil dependent chemiluminescence, *Crit. Care*, 2002, **6**, 342–348.
- 141 M. Nymark, P. J. Pussinen, A. M. Tuomainen, C. Forsblom, P. H. Groop, M. Lehto and FinnDiane Study Group, Serum lipopolysaccharide activity is associated with the progression



- of kidney disease in finnish patients with type 1 diabetes, *Diabetes Care*, 2009, **32**, 1689–1693.
- 142 O. S. Al-Attas, N. M. Al-Daghri, K. Al-Rubeaan, N. F. da Silva, S. L. Sabico, S. Kumar, P. G. McTernan and A. L. Harte, Changes in endotoxin levels in T2DM subjects on anti-diabetic therapies, *Cardiovasc. Diabetol.*, 2009, **8**, 20.
- 143 C. J. Wiedermann, S. Kiechl, S. Dunzendorfer, P. Schratzberger, G. Egger, F. Oberhollenzer and J. Willeit, Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck study, *J. Am. Coll. Cardiol.*, 1999, **34**, 1975–1981.
- 144 A. A. Nash, R. G. Dalziel and J. R. Fitzgerald, *Mims' pathogenesis of infectious disease*, Academic Press, New York, 6th edn, 2015.
- 145 L. Margulis and M. J. Chapman, Endosymbioses: cyclical and permanent in evolution, *Trends Microbiol.*, 1998, **6**, 342–345; discussion 345–346.
- 146 E. C. Cocking, P. J. Stone and M. R. Davey, Symbiosome-like intracellular colonization of cereals and other crop plants by nitrogen-fixing bacteria for reduced inputs of synthetic nitrogen fertilizers, *Sci. China, Ser. C: Life Sci.*, 2005, **48**(suppl 2), 888–896.
- 147 J. L. Knoth, S. H. Kim, G. J. Ettl and S. L. Doty, Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia, *New Phytol.*, 2014, **201**, 599–609.
- 148 B. Foxman, The epidemiology of urinary tract infection, *Nat. Rev. Urol.*, 2010, **7**, 653–660.
- 149 S. Heytens, A. De Sutter, D. De Backer, G. Verschraegen and T. Christiaens, Cystitis: symptomatology in women with suspected uncomplicated urinary tract infection, *J. Womens Health (Larchmt)*, 2011, **20**, 1117–1121.
- 150 J. Marschall, L. Zhang, B. Foxman, D. K. Warren, J. P. Henderson and CDC Prevention Epicenters Program, Both host and pathogen factors predispose to *Escherichia coli* urinary-source bacteremia in hospitalized patients, *Clin. Infect. Dis.*, 2012, **54**, 1692–1698.
- 151 G. J. Domingue, G. M. Ghoniem, K. L. Bost, C. Fermin and L. G. Human, Dormant microbes in interstitial cystitis, *J. Urol.*, 1995, **153**, 1321–1326.
- 152 B. Foxman, Recurring urinary tract infection: incidence and risk factors, *Am. J. Public Health*, 1990, **80**, 331–333.
- 153 B. Foxman, Epidemiology of urinary tract infections: incidence, morbidity, and economic costs, *Am. J. Med.*, 2002, **113**(suppl 1A), 5S–13S.
- 154 C. F. Marrs, L. Zhang and B. Foxman, *Escherichia coli* mediated urinary tract infections: are there distinct uropathogenic *E. coli* (UPEC) pathotypes?, *FEMS Microbiol. Lett.*, 2005, **252**, 183–190.
- 155 T. J. Hannan, I. U. Mysorekar, C. S. Hung, M. L. Isaacson-Schmid and S. J. Hultgren, Early severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection, *PLoS Pathog.*, 2010, **6**, e1001042.
- 156 D. A. Hunstad and S. S. Justice, Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*, *Annu. Rev. Microbiol.*, 2010, **64**, 203–221.
- 157 K. Ejrnaes, Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*, *Dan. Med. Bull.*, 2011, **58**, B4187.
- 158 T. J. Hannan, M. Totsika, K. J. Mansfield, K. H. Moore, M. A. Schembri and S. J. Hultgren, Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection, *FEMS Microbiol. Rev.*, 2012, **36**, 616–648.
- 159 B. Foxman, Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden, *Infectious disease clinics of North America*, 2014, **28**, 1–13.
- 160 A. Brauner, S. H. Jacobson and I. Kuhn, Urinary *Escherichia coli* causing recurrent infections – a prospective follow-up of biochemical phenotypes, *Clin. Nephrol.*, 1992, **38**, 318–323.
- 161 T. A. Russo, A. Stapleton, S. Wenderoth, T. M. Hooton and W. E. Stamm, Chromosomal restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women, *J. Infect. Dis.*, 1995, **172**, 440–445.
- 162 R. Ikäheimo, A. Siitonen, T. Heiskanen, U. Kärkkäinen, P. Kuosmanen, P. Lipponen and P. H. Mäkelä, Recurrence of urinary tract infection in a primary care setting: analysis of a 1-year follow-up of 179 women, *Clin. Infect. Dis.*, 1996, **22**, 91–99.
- 163 D. A. Rosen, J. S. Pinkner, J. M. Jones, J. N. Walker, S. Clegg and S. J. Hultgren, Utilization of an intracellular bacterial community pathway in *Klebsiella pneumoniae* urinary tract infection and the effects of FimK on type 1 pilus expression, *Infect. Immun.*, 2008, **76**, 3337–3345.
- 164 Y. Luo, Y. Ma, Q. Zhao, L. Wang, L. Guo, L. Ye, Y. Zhang and J. Yang, Similarity and divergence of phylogenies, antimicrobial susceptibilities, and virulence factor profiles of *Escherichia coli* isolates causing recurrent urinary tract infections that persist or result from reinfection, *J. Clin. Microbiol.*, 2012, **50**, 4002–4007.
- 165 J. M. Bower, D. S. Eto and M. A. Mulvey, Covert operations of uropathogenic *Escherichia coli* within the urinary tract, *Traffic*, 2005, **6**, 18–31.
- 166 S. S. Justice, C. Hung, J. A. Theriot, D. A. Fletcher, G. G. Anderson, M. J. Footer and S. J. Hultgren, Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1333–1338.
- 167 I. U. Mysorekar and S. J. Hultgren, Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 14170–14175.
- 168 D. A. Rosen, T. M. Hooton, W. E. Stamm, P. A. Humphrey and S. J. Hultgren, Detection of intracellular bacterial communities in human urinary tract infection, *PLoS Med.*, 2007, **4**, e329.
- 169 B. K. Dhakal, R. R. Kulesus and M. A. Mulvey, Mechanisms and consequences of bladder cell invasion by uropathogenic *Escherichia coli*, *Eur. J. Clin. Invest.*, 2008, **38**(suppl 2), 2–11.



- 170 D. J. Schwartz, S. L. Chen, S. J. Hultgren and P. C. Seed, Population dynamics and niche distribution of uropathogenic *Escherichia coli* during acute and chronic urinary tract infection, *Infect. Immun.*, 2011, **79**, 4250–4259.
- 171 X. R. Wu, T. T. Sun and J. J. Medina, *In vitro* binding of type 1-fimbriated *Escherichia coli* to uroplakins Ia and Ib: relation to urinary tract infections, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 9630–9635.
- 172 D. B. Kell, P. D. Dobson and S. G. Oliver, Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only, *Drug Discovery Today*, 2011, **16**, 704–714.
- 173 D. B. Kell, P. D. Dobson, E. Bilsland and S. G. Oliver, The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so, *Drug Discovery Today*, 2013, **18**, 218–239.
- 174 D. B. Kell and S. G. Oliver, How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion, *Front. Pharmacol.*, 2014, **5**, 231.
- 175 D. B. Kell, What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible?, *Trends Pharmacol. Sci.*, 2015, **36**, 15–21.
- 176 E. J. Arking, D. M. Appelt, J. T. Abrams, S. Kolbe, A. P. Hudson and B. J. Balin, Ultrastructural Analysis of *Chlamydia pneumoniae* in the Alzheimer's Brain, *Pathogenesis*, 1999, **1**, 201–211.
- 177 B. J. Balin, H. C. Gerard, E. J. Arking, D. M. Appelt, P. J. Branigan, J. T. Abrams, J. A. Whittum-Hudson and A. P. Hudson, Identification and localization of *Chlamydia pneumoniae* in the Alzheimer's brain, *Med. Microbiol. Immunol.*, 1998, **187**, 23–42.
- 178 B. J. Balin and D. M. Appelt, Role of infection in Alzheimer's disease, *J. Am. Osteopath. Assoc.*, 2001, **101**, S1–S6.
- 179 B. J. Balin, C. S. Little, C. J. Hammond, D. M. Appelt, J. A. Whittum-Hudson, H. C. Gerard and A. P. Hudson, *Chlamydia pneumoniae* and the etiology of late-onset Alzheimer's disease, *J. Alzheimer's Dis.*, 2008, **13**, 371–380.
- 180 H. C. Gérard, U. Dreses-Werringloer, K. S. Wildt, S. Deka, C. Oszust, B. J. Balin, W. H. Frey 2nd, E. Z. Bordayo, J. A. Whittum-Hudson and A. P. Hudson, *Chlamydia pneumoniae* in the Alzheimer's brain, *FEMS Immunol. Med. Microbiol.*, 2006, **48**, 355–366.
- 181 C. J. Hammond, L. R. Hallock, R. J. Howanski, D. M. Appelt, C. S. Little and B. J. Balin, Immunohistological detection of *Chlamydia pneumoniae* in the Alzheimer's disease brain, *BMC Neurosci.*, 2010, **11**, 121.
- 182 R. F. Itzhaki, M. A. Wozniak, D. M. Appelt and B. J. Balin, Infiltration of the brain by pathogens causes Alzheimer's disease, *Neurobiol. Aging*, 2004, **25**, 619–627.
- 183 C. Lim, C. J. Hammond, S. T. Hingley and B. J. Balin, *Chlamydia pneumoniae* infection of monocytes *in vitro* stimulates innate and adaptive immune responses relevant to those in Alzheimer's disease, *J. Neuroinflammation*, 2014, **11**, 5.
- 184 C. S. Little, C. J. Hammond, A. MacIntyre, B. J. Balin and D. M. Appelt, *Chlamydia pneumoniae* induces Alzheimer-like amyloid plaques in brains of BALB/c mice, *Neurobiol. Aging*, 2004, **25**, 419–429.
- 185 C. S. Little, T. A. Joyce, C. J. Hammond, H. Matta, D. Cahn, D. M. Appelt and B. J. Balin, Detection of bacterial antigens and Alzheimer's disease-like pathology in the central nervous system of BALB/c mice following intranasal infection with a laboratory isolate of *Chlamydia pneumoniae*, *Front. Aging Neurosci.*, 2014, **6**, 304.
- 186 C. B. Dobson, M. A. Wozniak and R. F. Itzhaki, Do infectious agents play a role in dementia?, *Trends Microbiol.*, 2003, **11**, 312–317.
- 187 R. F. Itzhaki and M. A. Wozniak, Alzheimer's disease, the neuroimmune axis, and viral infection, *J. Neuroimmunol.*, 2004, **156**, 1–2.
- 188 R. F. Itzhaki and M. A. Wozniak, Alzheimer's disease-like changes in herpes simplex virus type 1 infected cells: the case for antiviral therapy, *Rejuvenation Res.*, 2008, **11**, 319–320.
- 189 R. F. Itzhaki and M. A. Wozniak, Herpes simplex virus type 1 in Alzheimer's disease: the enemy within, *J. Alzheimer's Dis.*, 2008, **13**, 393–405.
- 190 R. F. Itzhaki, S. L. Cosby and M. A. Wozniak, Herpes simplex virus type 1 and Alzheimer's disease: the autophagy connection, *J. NeuroVirol.*, 2008, **14**, 1–4.
- 191 R. F. Itzhaki and M. A. Wozniak, Alzheimer's disease and infection: do infectious agents contribute to progression of Alzheimer's disease?, *Alzheimer's Dementia*, 2010, **6**, 83–84; author reply 85.
- 192 R. F. Itzhaki and M. A. Wozniak, Could antivirals be used to treat Alzheimer's disease?, *Future Microbiol.*, 2012, **7**, 307–309.
- 193 M. A. Wozniak and R. F. Itzhaki, Antiviral agents in Alzheimer's disease: hope for the future?, *Ther. Adv. Neurol. Disord.*, 2010, **3**, 141–152.
- 194 M. A. Wozniak, A. L. Frost, C. M. Preston and R. F. Itzhaki, Antivirals reduce the formation of key Alzheimer's disease molecules in cell cultures acutely infected with herpes simplex virus type 1, *PLoS One*, 2011, **6**, e25152.
- 195 M. A. Wozniak, A. L. Frost and R. F. Itzhaki, The helicase-primase inhibitor BAY 57-1293 reduces the Alzheimer's disease-related molecules induced by herpes simplex virus type 1, *Antiviral Res.*, 2013, **99**, 401–404.
- 196 J. Miklossy, Alzheimer's disease – a spirochetosis?, *NeuroReport*, 1993, **4**, 841–848.
- 197 J. Miklossy, S. Kasas, R. C. Janzer, F. Ardizzoni and H. Van der Loos, Further ultrastructural evidence that spirochaetes may play a role in the aetiology of Alzheimer's disease, *NeuroReport*, 1994, **5**, 1201–1204.
- 198 J. Miklossy, K. Khalili, L. Gern, R. L. Ericson, P. Darekar, L. Bolle, J. Hurlimann and B. J. Paster, *Borrelia burgdorferi* persists in the brain in chronic lyme neuroborreliosis and may be associated with Alzheimer disease, *J. Alzheimer's Dis.*, 2004, **6**, 639–649; discussion 673–681.
- 199 J. Miklossy, A. Kis, A. Radenovic, L. Miller, L. Forro, R. Martins, K. Reiss, N. Darbinian, P. Darekar, L. Mihaly



- and K. Khalili, Beta-amyloid deposition and Alzheimer's type changes induced by *Borrelia spirochetes*, *Neurobiol. Aging*, 2006, **27**, 228–236.
- 200 J. Miklossy, Chronic inflammation and amyloidogenesis in Alzheimer's disease – role of Spirochetes, *J. Alzheimer's Dis.*, 2008, **13**, 381–391.
- 201 J. Miklossy, Emerging roles of pathogens in Alzheimer disease, *Expert Rev. Mol. Med.*, 2011, **13**, e30.
- 202 J. Miklossy, Alzheimer's disease – a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria, *J. Neuroinflammation*, 2011, **8**, 90.
- 203 J. Miklossy, Historic evidence to support a causal relationship between spirochetal infections and Alzheimer's disease, *Front. Aging Neurosci.*, 2015, **7**, 46.
- 204 J. Miklossy, Biology and neuropathology of dementia in syphilis and Lyme disease, *Handb. Clin. Neurol.*, 2008, **89**, 825–844.
- 205 J. Miklossy, S. Kasas, A. D. Zurn, S. McCall, S. Yu and P. L. McGeer, Persisting atypical and cystic forms of *Borrelia burgdorferi* and local inflammation in Lyme neuroborreliosis, *J. Neuroinflammation*, 2008, **5**, 40.
- 206 J. Miklossy, Chronic or late lyme neuroborreliosis: analysis of evidence compared to chronic or late neurosyphilis, *Open Neurol. J.*, 2012, **6**, 146–157.
- 207 O. Miman, O. Y. Kusbeci, O. C. Aktepe and Z. Cetinkaya, The probable relation between *Toxoplasma gondii* and Parkinson's disease, *Neurosci. Lett.*, 2010, **475**, 129–131.
- 208 O. Y. Kusbeci, O. Miman, M. Yaman, O. C. Aktepe and S. Yazar, Could *Toxoplasma gondii* have any role in Alzheimer disease?, *Alzheimer Dis. Assoc. Disord.*, 2011, **25**, 1–3.
- 209 E. F. Torrey, J. J. Bartko and R. H. Yolken, *Toxoplasma gondii* and other risk factors for schizophrenia: an update, *Schizophr. Bull.*, 2012, **38**, 642–647.
- 210 R. Diaz Heijtz, S. Wang, F. Anuar, Y. Qian, B. Björkholm, A. Samuelsson, M. L. Hibberd, H. Forsberg and S. Pettersson, Normal gut microbiota modulates brain development and behavior, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 3047–3052.
- 211 J. F. Cryan and T. G. Dinan, Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour, *Nat. Rev. Neurosci.*, 2012, **13**, 701–712.
- 212 P. Forsythe, W. A. Kunze and J. Bienenstock, On communication between gut microbes and the brain, *Curr. Opin. Gastroenterol.*, 2012, **28**, 557–562.
- 213 X. Chen, R. D'Souza and S. T. Hong, The role of gut microbiota in the gut-brain axis: current challenges and perspectives, *Protein Cell*, 2013, **4**, 403–414.
- 214 J. A. Foster and K. A. McVey Neufeld, Gut-brain axis: how the microbiome influences anxiety and depression, *Trends Neurosci.*, 2013, **36**, 305–312.
- 215 M. Lyte, Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior, *PLoS Pathog.*, 2013, **9**, e1003726.
- 216 A. J. Montiel-Castro, R. M. González-Cervantes, G. Bravo-Ruiseco and G. Pacheco-López, The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality, *Front. Integr. Neurosci.*, 2013, **7**, 70.
- 217 P. Bercik and S. M. Collins, The effects of inflammation, infection and antibiotics on the microbiota-gut-brain axis, *Adv. Exp. Med. Biol.*, 2014, **817**, 279–289.
- 218 G. De Palma, S. M. Collins and P. Bercik, The microbiota-gut-brain axis in functional gastrointestinal disorders, *Gut Microbes*, 2014, **5**, 419–429.
- 219 G. De Palma, S. M. Collins, P. Bercik and E. F. Verdu, The microbiota-gut-brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both?, *J. Physiol.*, 2014, **592**, 2989–2997.
- 220 M. G. Gareau, Microbiota-gut-brain axis and cognitive function, *Adv. Exp. Med. Biol.*, 2014, **817**, 357–371.
- 221 P. Holzer and A. Farzi, Neuropeptides and the microbiota-gut-brain axis, *Adv. Exp. Med. Biol.*, 2014, **817**, 195–219.
- 222 M. Lyte, Microbial endocrinology and the microbiota-gut-brain axis, *Adv. Exp. Med. Biol.*, 2014, **817**, 3–24.
- 223 J. Bienenstock, W. Kunze and P. Forsythe, Microbiota and the gut-brain axis, *Nutr. Rev.*, 2015, **73**(suppl 1), 28–31.
- 224 M. Carabotti, A. Scirocco, M. A. Maselli and C. Severi, The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems, *Ann. Gastroenterol.*, 2015, **28**, 203–209.
- 225 X. Cong, W. A. Henderson, J. Graf and J. M. McGrath, Early Life Experience and Gut Microbiome: The Brain-Gut-Microbiota Signaling System, *Adv. Neonatal Care*, 2015, DOI: 10.1097/ANC.000000000000191.
- 226 R. A. Luna and J. A. Foster, Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression, *Curr. Opin. Biotechnol.*, 2015, **32**, 35–41.
- 227 E. A. Mayer, K. Tillisch and A. Gupta, Gut/brain axis and the microbiota, *J. Clin. Invest.*, 2015, **125**, 926–938.
- 228 V. Ridaura and Y. Belkaid, Gut microbiota: the link to your second brain, *Cell*, 2015, **161**, 193–194.
- 229 M. P. Sherman, H. Zaghouni and V. Niklas, Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis, *Pediatr. Res.*, 2015, **77**, 127–135.
- 230 S. L. Byler, G. W. Boehm, J. D. Karp, R. A. Kohman, A. J. Tarr, T. Schallert and T. M. Barth, Systemic lipopolysaccharide plus MPTP as a model of dopamine loss and gait instability in C57Bl/6J mice, *Behav. Brain Res.*, 2009, **198**, 434–439.
- 231 L. Hritcu, A. Ciobica, M. Stefan, M. Mihasan, L. Palamiuc and T. Nabeshima, Spatial memory deficits and oxidative stress damage following exposure to lipopolysaccharide in a rodent model of Parkinson's disease, *Neurosci. Res.*, 2011, **71**, 35–43.
- 232 L. Hritcu and A. Ciobica, Intranigral lipopolysaccharide administration induced behavioral deficits and oxidative stress damage in laboratory rats: relevance for Parkinson's disease, *Behav. Brain Res.*, 2013, **253**, 25–31.
- 233 M. Liu and G. Bing, Lipopolysaccharide animal models for Parkinson's disease, *Parkinson's Dis.*, 2011, **2011**, 327089.
- 234 Z. Zhang, K. Zhang, X. Du and Y. Li, Neuroprotection of desferrioxamine in lipopolysaccharide-induced nigrostriatal dopamine neuron degeneration, *Mol. Med. Rep.*, 2012, **5**, 562–566.



- 235 R. M. Santiago, J. Barbieiro, M. M. Lima, P. A. Dombrowski, R. Andreatini and M. A. B. F. Vital, Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2010, **34**, 1104–1114.
- 236 Q. He, W. Yu, J. Wu, C. Chen, Z. Lou, Q. Zhang, J. Zhao, J. Wang and B. Xiao, Intranasal LPS-mediated Parkinson's model challenges the pathogenesis of nasal cavity and environmental toxins, *PLoS One*, 2013, **8**, e78418.
- 237 D. B. Hoban, E. Connaughton, C. Connaughton, G. Hogan, C. Thornton, P. Mulcahy, T. C. Moloney and E. Dowd, Further characterisation of the LPS model of Parkinson's disease: a comparison of intra-nigral and intra-striatal lipopolysaccharide administration on motor function, microgliosis and nigrostriatal neurodegeneration in the rat, *Brain, Behav., Immun.*, 2013, **27**, 91–100.
- 238 C. B. Lawrence, D. Brough and E. M. Knight, Obese mice exhibit an altered behavioural and inflammatory response to lipopolysaccharide, *Dis. Models & Mech.*, 2012, **5**, 649–659.
- 239 J. Cohen, The immunopathogenesis of sepsis, *Nature*, 2002, **420**, 885–891.
- 240 C. Nathan, Points of control in inflammation, *Nature*, 2002, **420**, 846–852.
- 241 D. Annane, P. Aegerter, M. C. Jars-Guinestre, B. Guidet and CUB-Réa Network, Current epidemiology of septic shock: the CUB-Réa Network, *Am. J. Respir. Crit. Care Med.*, 2003, **168**, 165–172.
- 242 J. A. Russell, Management of sepsis, *N. Engl. J. Med.*, 2006, **355**, 1699–1713.
- 243 G. Suntharalingam, M. R. Perry, S. Ward, S. J. Brett, A. Castello-Cortes, M. D. Brunner and N. Panoskaltis, Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412, *N. Engl. J. Med.*, 2006, **355**, 1018–1028.
- 244 S. Sriskandan and D. M. Altmann, The immunology of sepsis, *J. Pathol.*, 2008, **214**, 211–223.
- 245 L. Ulloa, M. Brunner, L. Ramos and E. A. Deitch, Scientific and clinical challenges in sepsis, *Curr. Pharm. Des.*, 2009, **15**, 1918–1935.
- 246 N. R. London, W. Zhu, F. A. Bozza, M. C. Smith, D. M. Greif, L. K. Sorensen, L. Chen, Y. Kaminoh, A. C. Chan, S. F. Passi, C. W. Day, D. L. Barnard, G. A. Zimmerman, M. A. Krasnow and D. Y. Li, Targeting Robo4-dependent slit signaling to survive the cytokine storm in sepsis and influenza, *Sci. Transl. Med.*, 2010, **2**, 23ra19.
- 247 J. Hadem, C. Hafer, A. S. Schneider, O. Wiesner, G. Beutel, T. Fuehner, T. Welte, M. M. Hoepfer and J. T. Kielstein, Therapeutic plasma exchange as rescue therapy in severe sepsis and septic shock: retrospective observational single-centre study of 23 patients, *BMC Anesthesiol.*, 2014, **14**, 24.
- 248 M. M. Levy, M. P. Fink, J. C. Marshall, E. Abraham, D. Angus, D. Cook, J. Cohen, S. M. Opal, J. L. Vincent, G. Ramsay and Scm/Esicm/Accp/Ats/Sis, 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference, *Crit. Care Med.*, 2003, **31**, 1250–1256.
- 249 W. M. Dunne Jr., Laboratory diagnosis of sepsis? No SIRS, not just yet, *J. Clin. Microbiol.*, 2015, **53**, 2404–2409.
- 250 D. H. Lewis, D. L. Chan, D. Pinheiro, E. Armitage-Chan and O. A. Garden, The immunopathology of sepsis: pathogen recognition, systemic inflammation, the compensatory anti-inflammatory response, and regulatory T cells, *J. Vet. Intern. Med.*, 2012, **26**, 457–482.
- 251 F. Ratzinger, M. Schuardt, K. Eichbichler, I. Tsirkinidou, M. Bauer, H. Haslacher, D. Mitteregger, M. Binder and H. Burgmann, Utility of sepsis biomarkers and the infection probability score to discriminate sepsis and systemic inflammatory response syndrome in standard care patients, *PLoS One*, 2013, **8**, e82946.
- 252 R. P. Dellinger, M. M. Levy, A. Rhodes, D. Annane, H. Gerlach, S. M. Opal, J. E. Sevransky, C. L. Sprung, I. S. Douglas, R. Jaeschke, T. M. Osborn, M. E. Nunnally, S. R. Townsend, K. Reinhart, R. M. Kleinpell, D. C. Angus, C. S. Deutschman, F. R. Machado, G. D. Rubenfeld, S. A. Webb, R. J. Beale, J. L. Vincent, R. Moreno and Surviving Sepsis Campaign Guidelines Committee including the Pediatric Subgroup, Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012, *Crit. Care Med.*, 2013, **41**, 580–637.
- 253 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference, Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis, *Crit. Care Med.*, 1992, **20**, 864–874.
- 254 R. C. Bone, R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. Schein and W. J. Sibbald, Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine, *Chest*, 1992, **101**, 1644–1655.
- 255 G. S. Martin, D. M. Mannino and M. Moss, The effect of age on the development and outcome of adult sepsis, *Crit. Care Med.*, 2006, **34**, 15–21.
- 256 R. H. K. Eng, S. M. Smith, P. Fan-Havard and T. Ogbara, Effect of antibiotics on endotoxin release from Gram-negative bacteria, *Diagn. Microbiol. Infect. Dis.*, 1993, **16**, 185–189.
- 257 J. M. Prins, S. J. H. van Deventer, E. J. Kuijper and P. Speelman, Clinical relevance of antibiotic-induced endotoxin release, *Antimicrob. Agents Chemother.*, 1994, **38**, 1211–1218.
- 258 J. J. Jackson and H. Kropp, Differences in mode of action of beta-lactam antibiotics influence morphology, LPS release and *in vivo* antibiotic efficacy, *J. Endotoxin Res.*, 1996, **3**, 201–218.
- 259 D. C. Morrison, S. E. Bucklin, M. C. Leeson and M. Norimatsu, Contribution of soluble endotoxin released from Gram-negative bacteria by antibiotics to the pathogenesis of experimental sepsis in mice, *J. Endotoxin Res.*, 1996, **3**, 237–243.
- 260 T. Kirikae, M. Nakano and D. C. Morrison, Antibiotic-induced endotoxin release from bacteria and its clinical significance, *Microbiol. Immunol.*, 1997, **41**, 285–294.





- 261 R. G. Holzheimer, Antibiotic induced endotoxin release and clinical sepsis: a review, *J. Chemother.*, 2001, **13**(spec no. 1), 159–172.
- 262 P. M. Lepper, T. K. Held, E. M. Schneider, E. Bölke, H. Gerlach and M. Trautmann, Clinical implications of antibiotic-induced endotoxin release in septic shock, *Intensive Care Med.*, 2002, **28**, 824–833.
- 263 M. Trautmann, C. Scheibe, N. Wellinghausen, O. Holst and P. M. Lepper, Low endotoxin release from *Escherichia coli* and *Bacteroides fragilis* during exposure to moxifloxacin, *Chemotherapy*, 2010, **56**, 364–370.
- 264 S. M. Walters, V. S. Dubey, N. R. Jeffrey and D. R. Dixon, Antibiotic-induced *Porphyromonas gingivalis* LPS release and inhibition of LPS-stimulated cytokines by antimicrobial peptides, *Peptides*, 2010, **31**, 1649–1653.
- 265 S. Tanabe, M. Yoshioka, D. Hinode and D. Grenier, Sub-inhibitory concentrations of tetracyclines induce lipopolysaccharide shedding by *Porphyromonas gingivalis* and modulate the host inflammatory response, *J. Periodontal Res.*, 2014, **49**, 603–608.
- 266 G. Guerrier and E. D'Ortenzio, The Jarisch–Herxheimer reaction in leptospirosis: a systematic review, *PLoS One*, 2013, **8**, e59266.
- 267 P. Kadam, N. A. Gregory, B. Zelger and J. A. Carlson, Delayed Onset of the Jarisch–Herxheimer Reaction in Doxycycline-Treated Disease: a Case Report and Review of its Histopathology and Implications for Pathogenesis, *Am. J. Dermatopathol.*, 2015, **37**, e68–e74.
- 268 D. Fekade, K. Knox, K. Hussein, A. Melka, D. G. Lalloo, R. E. Coxon and D. A. Warrell, Prevention of Jarisch–Herxheimer reactions by treatment with antibodies against tumor necrosis factor alpha, *N. Engl. J. Med.*, 1996, **335**, 311–315.
- 269 P. W. Taylor, Bactericidal and bacteriolytic activity of serum against Gram-negative bacteria, *Microbiol. Rev.*, 1983, **47**, 46–83.
- 270 T. J. Iwashyna, E. W. Ely, D. M. Smith and K. M. Langa, Long-term cognitive impairment and functional disability among survivors of severe sepsis, *JAMA, J. Am. Med. Assoc.*, 2010, **304**, 1787–1794.
- 271 S. Yende, W. Linde-Zwirble, F. Mayr, L. A. Weissfeld, S. Reis and D. C. Angus, Risk of cardiovascular events in survivors of severe sepsis, *Am. J. Respir. Crit. Care Med.*, 2014, **189**, 1065–1074.
- 272 T. J. Iwashyna, C. R. Cooke, H. Wunsch and J. M. Kahn, Population burden of long-term survivorship after severe sepsis in older Americans, *J. Am. Geriatr. Soc.*, 2012, **60**, 1070–1077.
- 273 S. Yende, T. J. Iwashyna and D. C. Angus, Interplay between sepsis and chronic health, *Trends Mol. Med.*, 2014, **20**, 234–238.
- 274 M. R. Boocock and J. R. Coggins, Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate, *FEBS Lett.*, 1983, **154**, 127–133.
- 275 J. L. Rubin, C. G. Gaines and R. A. Jensen, Glyphosate inhibition of 5-enolpyruvylshikimate 3-phosphate synthase from suspension-cultured cells of *Nicotiana glauca*, *Plant Physiol.*, 1984, **75**, 839–845.
- 276 H. C. Steinrucken and N. Amrhein, 5-Enolpyruvylshikimate-3-phosphate synthase of *Klebsiella pneumoniae* 2. Inhibition by glyphosate [N-(phosphonomethyl)glycine], *Eur. J. Biochem.*, 1984, **143**, 351–357.
- 277 G. M. Kishore and D. M. Shah, Amino acid biosynthesis inhibitors as herbicides, *Annu. Rev. Biochem.*, 1988, **57**, 627–663.
- 278 A. Cornish-Bowden, Why is uncompetitive inhibition so rare? A possible explanation, with implications for the design of drugs and pesticides, *FEBS Lett.*, 1986, **203**, 3–6.
- 279 D. B. Kell, Metabolomics, modelling and machine learning in systems biology: towards an understanding of the languages of cells. The 2005 Theodor Bücher lecture, *FEBS J.*, 2006, **273**, 873–894.
- 280 A. Currin, N. Swainston, P. J. Day and D. B. Kell, Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently, *Chem. Soc. Rev.*, 2015, **44**, 1172–1239.
- 281 A. Goldbeter and D. E. Koshland Jr., An amplified sensitivity arising from covalent modification in biological systems, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 6840–6844.
- 282 A. Goldbeter and D. E. Koshland Jr., Ultrasensitivity in biochemical systems controlled by covalent modification. Interplay between zero-order and multistep effects, *J. Biol. Chem.*, 1984, **259**, 14441–14447.
- 283 M. L. Cárdenas and A. Cornish-Bowden, Characteristics necessary for an interconvertible enzyme cascade to generate a highly sensitive response to an effector, *Biochem. J.*, 1989, **257**, 339–345.
- 284 A. Cornish-Bowden, *Fundamentals of enzyme kinetics*, Portland Press, London, 2nd edn, 1995.
- 285 F. Ortega, L. Acerenza, H. V. Westerhoff, F. Mas and M. Cascante, Product dependence and bifunctionality compromise the ultrasensitivity of signal transduction cascades, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 1170–1175.
- 286 Y. Li and A. Goldbeter, Pulsatile signaling in intercellular communication. Periodic stimuli are more efficient than random or chaotic signals in a model based on receptor desensitization, *Biophys. J.*, 1992, **61**, 161–171.
- 287 L. Ashall, C. A. Horton, D. E. Nelson, P. Paszek, S. Ryan, K. Sillitoe, C. V. Harper, D. G. Spiller, J. F. Unitt, D. S. Broomhead, D. B. Kell, D. Rand, V. Sée and M. R. H. White, Pulsatile stimulation determines timing and specificity of NFκB-dependent transcription, *Science*, 2009, **324**, 242–246.
- 288 J. J. Tyson, K. C. Chen and B. Novak, Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell, *Curr. Opin. Cell Biol.*, 2003, **15**, 221–231.
- 289 D. B. Kell and J. D. Knowles, The role of modeling in systems biology, in *System modeling in cellular biology: from concepts to nuts and bolts*, ed. Z. Szallasi, J. Stelling and V. Periwal, MIT Press, Cambridge, 2006, pp. 3–18.
- 290 H. Y. Hsu and M. H. Wen, Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression, *J. Biol. Chem.*, 2002, **277**, 22131–22139.



- 291 C. A. Dinarello, Interleukin-1 in the pathogenesis and treatment of inflammatory diseases, *Blood*, 2011, **117**, 3720–3732.
- 292 C. A. Dinarello, A clinical perspective of IL-1beta as the gatekeeper of inflammation, *Eur. J. Immunol.*, 2011, **41**, 1203–1217.
- 293 S. U. Seo, N. Kamada, R. Munoz-Planillo, Y. G. Kim, D. Kim, Y. Koizumi, M. Hasegawa, S. D. Himpf, H. P. Browne, T. D. Lawley, H. L. Mobley, N. Inohara and G. Nunez, Distinct Commensals Induce Interleukin-1beta via NLRP3 Inflammation in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury, *Immunity*, 2015, **42**, 744–755.
- 294 J. R. Bethea, G. Y. Gillespie and E. N. Benveniste, Interleukin-1 beta induction of TNF-alpha gene expression: involvement of protein kinase C, *J. Cell. Physiol.*, 1992, **152**, 264–273.
- 295 A. L. Hopkins, J. S. Mason and J. P. Overington, Can we rationally design promiscuous drugs?, *Curr. Opin. Struct. Biol.*, 2006, **16**, 127–136.
- 296 A. L. Hopkins, Network pharmacology: the next paradigm in drug discovery, *Nat. Chem. Biol.*, 2008, **4**, 682–690.
- 297 D. B. Kell, Finding novel pharmaceuticals in the systems biology era using multiple effective drug targets, phenotypic screening, and knowledge of transporters: where drug discovery went wrong and how to fix it, *FEBS J.*, 2013, **280**, 5957–5980.
- 298 D. B. Kell and H. V. Westerhoff, Metabolic control theory: its role in microbiology and biotechnology, *FEMS Microbiol. Rev.*, 1986, **39**, 305–320.
- 299 A. Cornish-Bowden, J.-H. S. Hofmeyr and M. L. Cárdenas, Strategies for manipulating metabolic fluxes in biotechnology, *Bioorg. Chem.*, 1995, **23**, 439–449.
- 300 D. A. Fell, *Understanding the control of metabolism*, Portland Press, London, 1996.
- 301 D. A. Fell, Increasing the flux in metabolic pathways: a metabolic control analysis perspective, *Biotechnol. Bioeng.*, 1998, **58**, 121–124.
- 302 C. R. H. Raetz and C. Whitfield, Lipopolysaccharide endotoxins, *Annu. Rev. Biochem.*, 2002, **71**, 635–700.
- 303 M. Caroff and D. Karibian, Structure of bacterial lipopolysaccharides, *Carbohydr. Res.*, 2003, **338**, 2431–2447.
- 304 S. Müller-Loennies, L. Brade, C. R. MacKenzie, F. E. Di Padova and H. Brade, Identification of a cross-reactive epitope widely present in lipopolysaccharide from enterobacteria and recognized by the cross-protective monoclonal antibody WN1 222-5, *J. Biol. Chem.*, 2003, **278**, 25618–25627.
- 305 O. Holst, The structures of core regions from enterobacterial lipopolysaccharides – an update, *FEMS Microbiol. Lett.*, 2007, **271**, 3–11.
- 306 P. Swain, S. K. Nayak, P. K. Nanda and S. Dash, Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: a review, *Fish Shellfish Immunol.*, 2008, **25**, 191–201.
- 307 C. De Castro, M. Parrilli, O. Holst and A. Molinaro, Microbe-associated molecular patterns in innate immunity: extraction and chemical analysis of Gram-negative bacterial lipopolysaccharides, *Methods Enzymol.*, 2010, **480**, 89–115.
- 308 C. Whitfield, Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*, *Annu. Rev. Biochem.*, 2006, **75**, 39–68.
- 309 X. Wang and P. J. Quinn, Lipopolysaccharide: biosynthetic pathway and structure modification, *Prog. Lipid Res.*, 2010, **49**, 97–107.
- 310 E. Fahy, S. Subramaniam, H. A. Brown, C. K. Glass, A. H. Merrill Jr., R. C. Murphy, C. R. Raetz, D. W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. van Meer, M. S. VanNieuwenhze, S. H. White, J. L. Witztum and E. A. Dennis, A comprehensive classification system for lipids, *J. Lipid Res.*, 2005, **46**, 839–861.
- 311 C. R. H. Raetz, T. A. Garrett, C. M. Reynolds, W. A. Shaw, J. D. Moore, D. C. Smith Jr., A. A. Ribeiro, R. C. Murphy, R. J. Ulevitch, C. Fearn, D. Reichart, C. K. Glass, C. Benner, S. Subramaniam, R. Harkewicz, R. C. Bowers-Gentry, M. W. Buczynski, J. A. Cooper, R. A. Deems and E. A. Dennis, Kdo2-Lipid A of *Escherichia coli*, a defined endotoxin that activates macrophages via TLR-4, *J. Lipid Res.*, 2006, **47**, 1097–1111.
- 312 N. M. Kelly, L. Young and A. S. Cross, Differential induction of tumor necrosis factor by bacteria expressing rough and smooth lipopolysaccharide phenotypes, *Infect. Immun.*, 1991, **59**, 4491–4496.
- 313 E. Klipp, R. Herwig, A. Kowald, C. Wierling and H. Lehrach, *Systems biology in practice: concepts, implementation and clinical application*, Wiley/VCH, Berlin, 2005.
- 314 S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes and U. Kummer, COPASI: a COMplex PATHway Simulator, *Bioinformatics*, 2006, **22**, 3067–3074.
- 315 B. Ø. Palsson, *Systems biology: constraint-based reconstruction and analysis*, Cambridge University Press, Cambridge, 2015.
- 316 A. Nowotny, Relationship of structure and biological activity of bacterial endotoxins, *Naturwissenschaften*, 1971, **58**, 397–409.
- 317 E. T. Rietschel, T. Kirikae, F. U. Schade, U. Mamat, G. Schmidt, H. Loppnow, A. J. Ulmer, U. Zahring, U. Seydel and F. Di Padova, *et al.*, Bacterial endotoxin: molecular relationships of structure to activity and function, *FASEB J.*, 1994, **8**, 217–225.
- 318 S. I. Miller, R. K. Ernst and M. W. Bader, LPS, TLR4 and infectious disease diversity, *Nat. Rev. Microbiol.*, 2005, **3**, 36–46.
- 319 M. S. Trent, C. M. Stead, A. X. Tran and J. V. Hankins, Diversity of endotoxin and its impact on pathogenesis, *J. Endotoxin Res.*, 2006, **12**, 205–223.
- 320 Y. Tsutsumi-Ishii, K. Shimada, H. Daida, R. Toman and I. Nagaoka, Low potency of *Chlamydomonas* LPS to activate human mononuclear cells due to its reduced affinities for CD14 and LPS-binding protein, *Int. Immunol.*, 2008, **20**, 199–208.
- 321 J. C. Hurley, Diagnosis of endotoxemia with Gram-negative bacteremia is bacterial species dependent: a meta-analysis of clinical studies, *J. Clin. Microbiol.*, 2009, **47**, 3826–3831.
- 322 X. Wang and P. J. Quinn, Endotoxins: lipopolysaccharides of Gram-negative bacteria, *Subcell. Biochem.*, 2010, **53**, 3–25.



- 323 J. C. Hurley and S. M. Opal, Prognostic value of endotoxemia in patients with Gram-negative bacteremia is bacterial species dependent, *J. Innate Immun.*, 2013, **5**, 555–564.
- 324 C. Slocum, S. R. Coats, N. Hua, C. Kramer, G. Papadopoulos, E. O. Weinberg, C. V. Gudino, J. A. Hamilton, R. P. Darveau and C. A. Genco, Distinct lipid A moieties contribute to pathogen-induced site-specific vascular inflammation, *PLoS Pathog.*, 2014, **10**, e1004215.
- 325 M. P. Fink, Animal models of sepsis and its complications, *Kidney Int.*, 2008, **74**, 991–993.
- 326 M. P. Fink, Animal models of sepsis, *Virulence*, 2014, **5**, 143–153.
- 327 D. R. Webb, Animal models of human disease: inflammation, *Biochem. Pharmacol.*, 2014, **87**, 121–130.
- 328 D. E. Nelson, V. See, G. Nelson and M. R. White, Oscillations in transcription factor dynamics: a new way to control gene expression, *Biochem. Soc. Trans.*, 2004, **32**, 1090–1092.
- 329 A. E. C. Ihekweaba, D. S. Broomhead, R. Grimley, N. Benson, M. R. H. White and D. B. Kell, Synergistic control of oscillations in the NF- $\kappa$ B signalling pathway, *IEE Proc.: Syst. Biol.*, 2005, **152**, 153–160.
- 330 D. A. Turner, P. Paszek, D. J. Woodcock, D. E. Nelson, C. A. Horton, Y. Wang, D. G. Spiller, D. A. Rand, M. R. H. White and C. V. Harper, Physiological levels of TNF $\alpha$  stimulation induce stochastic dynamics of NF- $\kappa$ B responses in single living cells, *J. Cell Sci.*, 2010, **123**, 2834–2843.
- 331 G. Lahav, N. Rosenfeld, A. Sigal, N. Geva-Zatorsky, A. J. Levine, M. B. Elowitz and U. Alon, Dynamics of the p53-Mdm2 feedback loop in individual cells, *Nat. Genet.*, 2004, **36**, 147–150.
- 332 G. Lahav, Oscillations by the p53-Mdm2 feedback loop, *Adv. Exp. Med. Biol.*, 2008, **641**, 28–38.
- 333 E. Batchelor, A. Loewer, C. Mock and G. Lahav, Stimulus-dependent dynamics of p53 in single cells, *Mol. Syst. Biol.*, 2011, **7**, 488.
- 334 J. E. Purvis, K. W. Karhohs, C. Mock, E. Batchelor, A. Loewer and G. Lahav, p53 dynamics control cell fate, *Science*, 2012, **336**, 1440–1444.
- 335 D. Brough, R. A. Le Feuvre, Y. Iwakura and N. J. Rothwell, Purinergic (P2X7) receptor activation of microglia induces cell death *via* an interleukin-1-independent mechanism, *Mol. Cell. Neurosci.*, 2002, **19**, 272–280.
- 336 R. A. Le Feuvre, D. Brough, Y. Iwakura, K. Takeda and N. J. Rothwell, Priming of macrophages with lipopolysaccharide potentiates P2X7-mediated cell death *via* a caspase-1-dependent mechanism, independently of cytokine production, *J. Biol. Chem.*, 2002, **277**, 3210–3218.
- 337 M. E. Edye, G. Lopez-Castejon, S. M. Allan and D. Brough, Acidosis drives damage-associated molecular pattern (DAMP)-induced interleukin-1 secretion *via* a caspase-1-independent pathway, *J. Biol. Chem.*, 2013, **288**, 30485–30494.
- 338 A. J. Lee, K. J. Cho and J. H. Kim, MyD88-BLT2-dependent cascade contributes to LPS-induced interleukin-6 production in mouse macrophage, *Exp. Mol. Med.*, 2015, **47**, e156.
- 339 H. Mizutani, Y. Ishihara, A. Izawa, Y. Fujihara, S. Kobayashi, H. Gotou, E. Okabe, H. Takeda, Y. Ozawa, Y. Kamiya, H. Kamei, T. Kikuchi, G. Yamamoto, A. Mitani, T. Nishihara and T. Noguchi, Lipopolysaccharide of *Aggregatibacter actinomycetemcomitans* up-regulates inflammatory cytokines, prostaglandin E2 synthesis and osteoclast formation in interleukin-1 receptor antagonist-deficient mice, *J. Periodontol Res.*, 2013, **48**, 748–756.
- 340 A. Meng, X. Zhang and Y. Shi, Role of p38 MAPK and STAT3 in lipopolysaccharide-stimulated mouse alveolar macrophages, *Exp. Ther. Med.*, 2014, **8**, 1772–1776.
- 341 D. Brough, P. Pelegrin and N. J. Rothwell, Pannexin-1-dependent caspase-1 activation and secretion of IL-1 beta is regulated by zinc, *Eur. J. Immunol.*, 2009, **39**, 352–358.
- 342 M. Lee, S. H. Kim, H. K. Lee, Y. Cho, J. Kang and S. H. Sung, ent-kaurane and ent-pimarane diterpenes from *Siegesbeckia pubescens* inhibit lipopolysaccharide-induced nitric oxide production in BV2 microglia, *Biol. Pharm. Bull.*, 2014, **37**, 152–157.
- 343 B. G. Small, B. W. McColl, R. Allmendinger, R. Pahle, G. Lopez-Castejon, N. J. Rothwell, J. Knowles, P. Mendes, D. Brough and D. B. Kell, Efficient discovery of anti-inflammatory small molecule combinations using evolutionary computing, *Nat. Chem. Biol.*, 2011, **7**, 902–908.
- 344 B. G. Taksande, C. T. Chopde, M. J. Umekar and N. R. Kotagale, Agmatine attenuates lipopolysaccharide induced anorexia and sickness behavior in rats, *Pharmacol., Biochem. Behav.*, 2015, **132**, 108–114.
- 345 F. Gao, Z. Liu, W. Ren and W. Jiang, Acute lipopolysaccharide exposure facilitates epileptiform activity *via* enhanced excitatory synaptic transmission and neuronal excitability *in vitro*, *Neuropsychiatr. Dis. Treat.*, 2014, **10**, 1489–1495.
- 346 K. Chatzivasilieiou, C. A. Lux, G. Steinhoff and H. Lang, Dental follicle progenitor cells responses to *Porphyromonas gingivalis* LPS, *J. Cell. Mol. Med.*, 2013, **17**, 766–773.
- 347 S. Braesch-Andersen, S. Paulie, C. Smedman, S. Mia and M. Kumagai-Braesch, ApoE production in human monocytes and its regulation by inflammatory cytokines, *PLoS One*, 2013, **8**, e79908.
- 348 J. D. Rempel, J. Packiasamy, H. J. Dean, J. McGavock, A. Janke, M. Collister, B. Wicklow and E. A. Sellers, Preliminary analysis of immune activation in early onset type 2 diabetes, *Int. J. Circumpolar Health*, 2013, **72**, 21190.
- 349 L. Fernández-Bertolín, J. Mullol, M. Fuentes-Prado, J. Roca-Ferrer, I. Alobid, C. Picado and L. Pujols, Effect of lipopolysaccharide on glucocorticoid receptor function in control nasal mucosa fibroblasts and in fibroblasts from patients with chronic rhinosinusitis with nasal polyps and asthma, *PLoS One*, 2015, **10**, e0125443.
- 350 C. Sandersen, D. Bienzle, S. Cerri, T. Franck, S. Derocette, P. Neven, A. Mouytis-Mickalad and D. SerTEyn, Effect of inhaled hydrosoluble curcumin on inflammatory markers in broncho-alveolar lavage fluid of horses with LPS-induced lung neutrophilia, *Multidiscip. Respir. Med.*, 2015, **10**, 16.
- 351 D. Lamarque, A. P. Moran, Z. Szepes, J. C. Delchier and B. J. Whittle, Cytotoxicity associated with induction of



- nitric oxide synthase in rat duodenal epithelial cells *in vivo* by lipopolysaccharide of *Helicobacter pylori*: inhibition by superoxide dismutase, *Br. J. Pharmacol.*, 2000, **130**, 1531–1538.
- 352 K. L. Comstock, K. A. Krown, M. T. Page, D. Martin, P. Ho, M. Pedraza, E. N. Castro, N. Nakajima, C. C. Glembotski, P. J. E. Quintana and R. A. Sabbadini, LPS-Induced TNF- $\alpha$  release from and apoptosis in rat cardiomyocytes: Obligatory role for CD14 in mediating the LPS response, *J. Mol. Cell. Cardiol.*, 1998, **30**, 2761–2775.
- 353 Q. Xiong, Q. Ru, L. Chen, K. Yue, X. Tian, B. Ma, L. Liu, R. Wu, C. Xu, M. Pi and C. Li, Combined Effects of Fine Particulate Matter and Lipopolysaccharide on Apoptotic Responses in NR8383 Macrophages, *J. Toxicol. Environ. Health, Part A*, 2015, **78**, 443–452.
- 354 P. C. Li, Y. C. Tien, C. H. Day, P. Pai, W. W. Kuo, T. S. Chen, C. H. Kuo, C. H. Tsai, D. T. Ju and C. Y. Huang, Impact of LPS-induced cardiomyoblast cell apoptosis inhibited by earthworm extracts, *Cardiovasc. Toxicol.*, 2015, **15**, 172–179.
- 355 L. Zhong, X. L. Zhou, Y. S. Liu, Y. M. Wang, F. Ma, B. L. Guo, Z. Q. Yan and Q. Y. Zhang, Estrogen receptor  $\alpha$  mediates the effects of notoginsenoside R1 on endotoxin-induced inflammatory and apoptotic responses in H9c2 cardiomyocytes, *Mol. Med. Rep.*, 2015, **12**, 119–126.
- 356 C. Guo, L. Yuan, J. G. Wang, F. Wang, X. K. Yang, F. H. Zhang, J. L. Song, X. Y. Ma, Q. Cheng and G. H. Song, Lipopolysaccharide (LPS) induces the apoptosis and inhibits osteoblast differentiation through JNK pathway in MC3T3-E1 cells, *Inflammation*, 2014, **37**, 621–631.
- 357 M. Takahashi, A. Ota, S. Karnan, E. Hossain, Y. Konishi, L. Damdindorj, H. Konishi, T. Yokochi, M. Nitta and Y. Hosokawa, Arsenic trioxide prevents nitric oxide production in lipopolysaccharide – stimulated RAW 264.7 by inhibiting a TRIF-dependent pathway, *Cancer Sci.*, 2013, **104**, 165–170.
- 358 J. Xaus, M. Comalada, A. F. Valledor, J. Lloberas, F. López-Soriano, J. M. Argilés, C. Bogdan and A. Celada, LPS induces apoptosis in macrophages mostly through the autocrine production of TNF- $\alpha$ , *Blood*, 2000, **95**, 3823–3831.
- 359 J. Zhao, X. Li, M. Zou, J. He, Y. Han, D. Wu, H. Yang and J. Wu, miR-135a inhibition protects A549 cells from LPS-induced apoptosis by targeting Bcl-2, *Biochem. Biophys. Res. Commun.*, 2014, **452**, 951–957.
- 360 A. L. Blomkalns, L. L. Stoll, W. Shaheen, S. A. Romig-Martin, E. W. Dickson, N. L. Weintraub and G. M. Denning, Low level bacterial endotoxin activates two distinct signaling pathways in human peripheral blood mononuclear cells, *J. Inflammation*, 2011, **8**, 4.
- 361 X. J. Dai, N. Li, L. Yu, Z. Y. Chen, R. Hua, X. Qin and Y. M. Zhang, Activation of BV2 microglia by lipopolysaccharide triggers an inflammatory reaction in PC12 cell apoptosis through a toll-like receptor 4-dependent pathway, *Cell Stress Chaperones*, 2015, **20**, 321–331.
- 362 J. Li, J. He and C. Yu, Chitosan oligosaccharide inhibits LPS-induced apoptosis of vascular endothelial cells through the BKCa channel and the p38 signaling pathway, *Int. J. Mol. Med.*, 2012, **30**, 157–164.
- 363 I. S. Kim, H. M. Ko, S. Koppula, B. W. Kim and D. K. Choi, Protective effect of *Chrysanthemum indicum* Linne against 1-methyl-4-phenylpyridinium ion and lipopolysaccharide-induced cytotoxicity in cellular model of Parkinson's disease, *Food Chem. Toxicol.*, 2011, **49**, 963–973.
- 364 P. Matzinger, The danger model: a renewed sense of self, *Science*, 2002, **296**, 301–305.
- 365 T. Pradeu and E. L. Cooper, The danger theory: 20 years later, *Front. Immunol.*, 2012, **3**, 287.
- 366 C. A. Janeway Jr., Approaching the asymptote? Evolution and revolution in immunology, *Cold Spring Harbor Symp. Quant. Biol.*, 1989, **54**(pt 1), 1–13.
- 367 D. L. Tang, R. Kang, C. B. Coyne, H. J. Zeh and M. T. Lotze, PAMPs and DAMPs: signal 0s that spur autophagy and immunity, *Immunol. Rev.*, 2012, **249**, 158–175.
- 368 A. Poltorak, X. L. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton and B. Beutler, Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene, *Science*, 1998, **282**, 2085–2088.
- 369 A. Poltorak, P. Ricciardi-Castagnoli, S. Citterio and B. Beutler, Physical contact between lipopolysaccharide and Toll-like receptor 4 revealed by genetic complementation, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 2163–2167.
- 370 B. Beutler, X. Du and A. Poltorak, Identification of Toll-like receptor 4 (Tlr4) as the sole conduit for LPS signal transduction: genetic and evolutionary studies, *J. Endotoxin Res.*, 2001, **7**, 277–280.
- 371 Y. C. Lu, W. C. Yeh and P. S. Ohashi, LPS/TLR4 signal transduction pathway, *Cytokine*, 2008, **42**, 145–151.
- 372 B. S. Park, D. H. Song, H. M. Kim, B. S. Choi, H. Lee and J. O. Lee, The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex, *Nature*, 2009, **458**, 1191–1195.
- 373 L. A. J. O'Neill, D. Golenbock and A. G. Bowie, The history of Toll-like receptors – redefining innate immunity, *Nat. Rev. Immunol.*, 2013, **13**, 453–460.
- 374 K. Fassbender, S. Walter, S. Kuhl, R. Landmann, K. Ishii, T. Bertsch, A. K. Stalder, F. Muehlhauser, Y. Liu, A. J. Ulmer, S. Rivest, A. Lentschat, E. Gulbins, M. Jucker, M. Staufenbiel, K. Brechtel, J. Walter, G. Multhaup, B. Penke, Y. Adachi, T. Hartmann and K. Beyreuther, The LPS receptor (CD14) links innate immunity with Alzheimer's disease, *FASEB J.*, 2004, **18**, 203–205.
- 375 Y. Liu, S. Walter, M. Stagi, D. Cherny, M. Letiembre, W. Schulz-Schaeffer, H. Heine, B. Penke, H. Neumann and K. Fassbender, LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide, *Brain*, 2005, **128**, 1778–1789.
- 376 E. G. Reed-Geaghan, J. C. Savage, A. G. Hise and G. E. Landreth, CD14 and toll-like receptors 2 and 4 are required for fibrillar A $\beta$ -stimulated microglial activation, *J. Neurosci.*, 2009, **29**, 11982–11992.



- 377 F. L. Heppner, R. M. Ransohoff and B. Becher, Immune attack: the role of inflammation in Alzheimer disease, *Nat. Rev. Neurosci.*, 2015, **16**, 358–372.
- 378 L. Verstrepen, T. Bekaert, T. L. Chau, J. Tavernier, A. Chariot and R. Beyaert, TLR-4, IL-1R and TNF-R signaling to NF-kappa B: variations on a common theme, *Cell. Mol. Life Sci.*, 2008, **65**, 2964–2978.
- 379 L. A. J. O'Neill, C. E. Bryant and S. L. Doyle, Therapeutic targeting of toll-like receptors for infectious and inflammatory diseases and cancer, *Pharmacol. Rev.*, 2009, **61**, 177–197.
- 380 O. Takeuchi and S. Akira, Pattern Recognition Receptors and Inflammation, *Cell*, 2010, **140**, 805–820.
- 381 T. Kawai and S. Akira, Toll-like receptors and their cross-talk with other innate receptors in infection and immunity, *Immunity*, 2011, **34**, 637–650.
- 382 P. P. Tak and G. S. Firestein, NF-kappaB: a key role in inflammatory diseases, *J. Clin. Invest.*, 2001, **107**, 7–11.
- 383 J. Ding, D. Song, X. Ye and S. F. Liu, A pivotal role of endothelial-specific NF-kappaB signaling in the pathogenesis of septic shock and septic vascular dysfunction, *J. Immunol.*, 2009, **183**, 4031–4038.
- 384 S. Vallabhapurapu and M. Karin, Regulation and function of NF-kappaB transcription factors in the immune system, *Annu. Rev. Immunol.*, 2009, **27**, 693–733.
- 385 A. R. Noort, P. P. Tak and S. W. Tas, Non-canonical NF-kappaB signaling in rheumatoid arthritis: Dr Jekyll and Mr Hyde?, *Arthritis Res. Ther.*, 2015, **17**, 15.
- 386 J. A. Kellum, L. Kong, M. P. Fink, L. A. Weissfeld, D. M. Yealy, M. R. Pinsky, J. Fine, A. Krichevsky, R. L. Delude, D. C. Angus and I. M. S. I. Gen, Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study, *Arch. Intern. Med.*, 2007, **167**, 1655–1663.
- 387 J. A. Hagar, D. A. Powell, Y. Aachoui, R. K. Ernst and E. A. Miao, Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock, *Science*, 2013, **341**, 1250–1253.
- 388 N. Kayagaki, M. T. Wong, I. B. Stowe, S. R. Ramani, L. C. Gonzalez, S. Akashi-Takamura, K. Miyake, J. Zhang, W. P. Lee, A. Muszynski, L. S. Forsberg, R. W. Carlson and V. M. Dixit, Noncanonical Inflammasome Activation by Intracellular LPS Independent of TLR4, *Science*, 2013, **341**, 1246–1249.
- 389 J. Shi, Y. Zhao, Y. Wang, W. Gao, J. Ding, P. Li, L. Hu and F. Shao, Inflammatory caspases are innate immune receptors for intracellular LPS, *Nature*, 2014, **514**, 187–192.
- 390 A. Denes, G. Coutts, N. Lénárt, S. M. Cruickshank, P. Pelegrin, J. Skinner, N. Rothwell, S. M. Allan and D. Brough, AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 4050–4055.
- 391 E. Latz, T. S. Xiao and A. Stutz, Activation and regulation of the inflammasomes, *Nat. Rev. Immunol.*, 2013, **13**, 397–411.
- 392 M. Chamaillard, M. Hashimoto, Y. Horie, J. Masumoto, S. Qiu, L. Saab, Y. Ogura, A. Kawasaki, K. Fukase, S. Kusumoto, M. A. Valvano, S. J. Foster, T. W. Mak, G. Nuñez and N. Inohara, An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid, *Nat. Immunol.*, 2003, **4**, 702–707.
- 393 M. Chamaillard, N. Inohara and G. Nuñez, Battling enteroinvasive bacteria: Nod1 comes to the rescue, *Trends Microbiol.*, 2004, **12**, 529–532.
- 394 S. E. Girardin, I. G. Boneca, J. Viala, M. Chamaillard, A. Labigne, G. Thomas, D. J. Philpott and P. J. Sansonetti, Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection, *J. Biol. Chem.*, 2003, **278**, 8869–8872.
- 395 S. E. Girardin, I. G. Boneca, L. A. Carneiro, A. Antignac, M. Jéhanno, J. Viala, K. Tedin, M. K. Taha, A. Labigne, U. Zähringer, A. J. Coyle, P. S. DiStefano, J. Bertin, P. J. Sansonetti and D. J. Philpott, Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan, *Science*, 2003, **300**, 1584–1587.
- 396 S. Traub, N. Kubasch, S. Morath, M. Kresse, T. Hartung, R. R. Schmidt and C. Hermann, Structural requirements of synthetic muropeptides to synergize with lipopolysaccharide in cytokine induction, *J. Biol. Chem.*, 2004, **279**, 8694–8700.
- 397 I. G. Boneca, The role of peptidoglycan in pathogenesis, *Curr. Opin. Microbiol.*, 2005, **8**, 46–53.
- 398 I. Tattoli, L. H. Travassos, L. A. Carneiro, J. G. Magalhaes and S. E. Girardin, The Nodosome: Nod1 and Nod2 control bacterial infections and inflammation, *Semin. Immunopathol.*, 2007, **29**, 289–301.
- 399 L. Le Bourhis, S. Benko and S. E. Girardin, Nod1 and Nod2 in innate immunity and human inflammatory disorders, *Biochem. Soc. Trans.*, 2007, **35**, 1479–1484.
- 400 M. A. Boudreau, J. F. Fisher and S. Mobashery, Messenger functions of the bacterial cell wall-derived muropeptides, *Biochemistry*, 2012, **51**, 2974–2990.
- 401 W. Deng and J. Xie, NOD2 signaling and role in pathogenic mycobacterium recognition, infection and immunity, *Cell. Physiol. Biochem.*, 2012, **30**, 953–963.
- 402 J. W. Johnson, J. F. Fisher and S. Mobashery, Bacterial cell-wall recycling, *Ann. N. Y. Acad. Sci.*, 2013, **1277**, 54–75.
- 403 J. Dworkin, The medium is the message: interspecies and interkingdom signaling by peptidoglycan and related bacterial glycans, *Annu. Rev. Microbiol.*, 2014, **68**, 137–154.
- 404 M. S. Siegrist, B. M. Swarts, D. M. Fox, S. A. Lim and C. R. Bertozzi, Illumination of growth, division and secretion by metabolic labeling of the bacterial cell surface, *FEMS Microbiol. Rev.*, 2015, **39**, 184–202.
- 405 W. Strober, P. J. Murray, A. Kitani and T. Watanabe, Signalling pathways and molecular interactions of NOD1 and NOD2, *Nat. Rev. Immunol.*, 2006, **6**, 9–20.
- 406 K. Kobayashi, N. Inohara, L. D. Hernandez, J. E. Galan, G. Nunez, C. A. Janeway, R. Medzhitov and R. A. Flavell, RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems, *Nature*, 2002, **416**, 194–199.
- 407 J. G. Magalhaes, J. Lee, K. Geddes, S. Rubino, D. J. Philpott and S. E. Girardin, Essential role of Rip2 in the modulation



- of innate and adaptive immunity triggered by Nod1 and Nod2 ligands, *Eur. J. Immunol.*, 2011, **41**, 1445–1455.
- 408 G. V. Mukamolova, A. G. Murzin, E. G. Salina, G. R. Demina, D. B. Kell, A. S. Kaprelyants and M. Young, Muralytic activity of *Micrococcus luteus* Rpf and its relationship to physiological activity in promoting bacterial growth and resuscitation, *Mol. Microbiol.*, 2006, **59**, 84–98.
- 409 N. H. Keep, J. M. Ward, M. Cohen-Gonsaud and B. Henderson, Wake up! Peptidoglycan lysis and bacterial non-growth states, *Trends Microbiol.*, 2006, **14**, 271–276.
- 410 D. E. Smith, B. Cléménçon and M. A. Hediger, Proton-coupled oligopeptide transporter family SLC15: physiological, pharmacological and pathological implications, *Mol. Aspects Med.*, 2013, **34**, 323–336.
- 411 R. S. Hotchkiss and I. E. Karl, The pathophysiology and treatment of sepsis, *N. Engl. J. Med.*, 2003, **348**, 138–150.
- 412 L. Hoareau, K. Bencharif, P. Rondeau, R. Murumalla, P. Ravanan, F. Tallet, P. Delarue, M. Cesari, R. Roche and F. Festy, Signaling pathways involved in LPS induced TNF $\alpha$  production in human adipocytes, *J. Inflammation*, 2010, **7**, 1.
- 413 Y. Kobayashi, A. Iwata, K. Suzuki, A. Suto, S. Kawashima, Y. Saito, T. Owada, M. Kobayashi, N. Watanabe and H. Nakajima, B and T lymphocyte attenuator inhibits LPS-induced endotoxic shock by suppressing Toll-like receptor 4 signaling in innate immune cells, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 5121–5126.
- 414 L. Koch, D. Frommhold, K. Buschmann, N. Kuss, J. Poeschl and P. Ruef, LPS- and LTA-induced expression of IL-6 and TNF- $\alpha$  in neonatal and adult blood: role of MAPKs and NF- $\kappa$ B, *Mediators Inflammation*, 2014, **2014**, 283126.
- 415 T. Wada and J. M. Penninger, Mitogen-activated protein kinases in apoptosis regulation, *Oncogene*, 2004, **23**, 2838–2849.
- 416 N. D. Perkins, NF- $\kappa$ B: tumor promoter or suppressor?, *Trends Cell Biol.*, 2004, **14**, 64–69.
- 417 N. D. Perkins, Integrating cell-signalling pathways with NF- $\kappa$ B and IKK function, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 49–62.
- 418 Y. Ben-Neriah and M. Karin, Inflammation meets cancer, with NF- $\kappa$ B as the matchmaker, *Nat. Immunol.*, 2011, **12**, 715–723.
- 419 M. Guma, D. Stepniak, H. Shaked, M. E. Spehlmann, S. Shenouda, H. Cheroutre, I. Vicente-Suarez, L. Eckmann, M. F. Kagnoff and M. Karin, Constitutive intestinal NF- $\kappa$ B does not trigger destructive inflammation unless accompanied by MAPK activation, *J. Exp. Med.*, 2011, **208**, 1889–1900.
- 420 J. J. Haddad and N. E. Abdel-Karim, NF- $\kappa$ B cellular and molecular regulatory mechanisms and pathways: therapeutic pattern or pseudoregulation?, *Cell. Immunol.*, 2011, **271**, 5–14.
- 421 Y. Wang, P. Paszek, C. A. Horton, D. B. Kell, M. R. H. White, D. S. Broomhead and M. R. Muldoon, Interactions among oscillatory pathways in NF- $\kappa$ B signalling, *BMC Syst. Biol.*, 2011, **5**, 23.
- 422 J. A. DiDonato, F. Mercurio and M. Karin, NF- $\kappa$ B and the link between inflammation and cancer, *Immunol. Rev.*, 2012, **246**, 379–400.
- 423 N. D. Perkins, The diverse and complex roles of NF- $\kappa$ B subunits in cancer, *Nat. Rev. Cancer*, 2012, **12**, 121–132.
- 424 Y. Wang, P. Paszek, C. A. Horton, H. Yue, M. R. H. White, D. B. Kell, M. R. Muldoon and D. S. Broomhead, A systematic survey of the response of a model NF- $\kappa$ B signalling pathway to TNF $\alpha$  stimulation, *J. Theor. Biol.*, 2012, **297**, 137–147.
- 425 F. Pinheiro da Silva and V. Nizet, Cell death during sepsis: integration of disintegration in the inflammatory response to overwhelming infection, *Apoptosis*, 2009, **14**, 509–521.
- 426 D. Moquin and F. K. Chan, The molecular regulation of programmed necrotic cell injury, *Trends Biochem. Sci.*, 2010, **35**, 434–441.
- 427 F. K. Chan, Fueling the flames: mammalian programmed necrosis in inflammatory diseases, *Cold Spring Harbor Perspect. Biol.*, 2012, **4**, a008805.
- 428 S. Ardestani, D. L. Deskins and P. P. Young, Membrane TNF- $\alpha$ -activated programmed necrosis is mediated by Ceramide-induced reactive oxygen species, *J. Mol. Signaling*, 2013, **8**, 12.
- 429 G. W. Dorn, 2nd, Molecular mechanisms that differentiate apoptosis from programmed necrosis, *Toxicol. Pathol.*, 2013, **41**, 227–234.
- 430 M. Dagenais, T. Douglas and M. Saleh, Role of programmed necrosis and cell death in intestinal inflammation, *Curr. Opin. Gastroenterol.*, 2014, **30**, 566–575.
- 431 A. Degterev, W. Zhou, J. L. Maki and J. Yuan, Assays for necroptosis and activity of RIP kinases, *Methods Enzymol.*, 2014, **545**, 1–33.
- 432 H. Sridharan and J. W. Upton, Programmed necrosis in microbial pathogenesis, *Trends Microbiol.*, 2014, **22**, 199–207.
- 433 L. Sun and X. Wang, A new kind of cell suicide: mechanisms and functions of programmed necrosis, *Trends Biochem. Sci.*, 2014, **39**, 587–593.
- 434 F. K. Chan, N. F. Luz and K. Moriwaki, Programmed necrosis in the cross talk of cell death and inflammation, *Annu. Rev. Immunol.*, 2015, **33**, 79–106.
- 435 M. Feoktistova and M. Leverkus, Programmed necrosis and necroptosis signalling, *FEBS J.*, 2015, **282**, 19–31.
- 436 S. Jouan-Lanhouet, F. Riquet, L. Duprez, T. Vanden Berghe, N. Takahashi and P. Vandenabeele, Necroptosis, *in vivo* detection in experimental disease models, *Semin. Cell Dev. Biol.*, 2014, **35**, 2–13.
- 437 M. Pasparakis and P. Vandenabeele, Necroptosis and its role in inflammation, *Nature*, 2015, **517**, 311–320.
- 438 S. L. Fink and B. T. Cookson, Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells, *Infect. Immun.*, 2005, **73**, 1907–1916.
- 439 S. L. Fink and B. T. Cookson, Pyroptosis and host cell death responses during *Salmonella* infection, *Cell. Microbiol.*, 2007, **9**, 2562–2570.
- 440 H. Ashida, H. Mimuro, M. Ogawa, T. Kobayashi, T. Sanada, M. Kim and C. Sasakawa, Cell death and infection:



- a double-edged sword for host and pathogen survival, *J. Cell Biol.*, 2011, **195**, 931–942.
- 441 K. Labbé and M. Saleh, Pyroptosis: A Caspase-1-Dependent Programmed Cell Death and a Barrier to Infection, *Prog. Inflammation Res.*, 2011, 17–36.
- 442 T. Bergsbaken, S. L. Fink and B. T. Cookson, Pyroptosis: host cell death and inflammation, *Nat. Rev. Microbiol.*, 2009, **7**, 99–109.
- 443 K. R. Bortoluci and R. Medzhitov, Control of infection by pyroptosis and autophagy: role of TLR and NLR, *Cell. Mol. Life Sci.*, 2010, **67**, 1643–1651.
- 444 O. Kepp, L. Galluzzi, L. Zitvogel and G. Kroemer, Pyroptosis – a cell death modality of its kind?, *Eur. J. Immunol.*, 2010, **40**, 627–630.
- 445 P. Vandenamee, L. Galluzzi, T. Vanden Berghe and G. Kroemer, Molecular mechanisms of necroptosis: an ordered cellular explosion, *Nat. Rev. Mol. Cell Biol.*, 2010, **11**, 700–714.
- 446 C. R. Lupfer and T. D. Kanneganti, The role of inflammasome modulation in virulence, *Virulence*, 2012, **3**, 262–270.
- 447 Y. Achoui, V. Sagulenko, E. A. Miao and K. J. Stacey, Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection, *Curr. Opin. Microbiol.*, 2013, **16**, 319–326.
- 448 L. D. Cunha and D. S. Zamboni, Subversion of inflammasome activation and pyroptosis by pathogenic bacteria, *Front. Cell. Infect. Microbiol.*, 2013, **3**, 76.
- 449 G. Doitsh, N. L. Galloway, X. Geng, Z. Yang, K. M. Monroe, O. Zepeda, P. W. Hunt, H. Hatano, S. Sowinski, I. Muñoz-Arias and W. C. Greene, Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection, *Nature*, 2013, **505**, 509–514.
- 450 C. N. LaRock and B. T. Cookson, Burning down the house: cellular actions during pyroptosis, *PLoS Pathog.*, 2013, **9**, e1003793.
- 451 S. J. Dixon, K. M. Lemberg, M. R. Lamprecht, R. Skouta, E. M. Zaitsev, C. E. Gleason, D. N. Patel, A. J. Bauer, A. M. Cantley, W. S. Yang, B. Morrison 3rd and B. R. Stockwell, Ferroptosis: an iron-dependent form of nonapoptotic cell death, *Cell*, 2012, **149**, 1060–1072.
- 452 P. Guan, Z. H. Shi, Y. Q. Li and N. Wang, Ferroptosis: a new mechanism of cell death, *Prog. Biochem. Biophys.*, 2013, **40**, 137–140.
- 453 S. Toyokuni, Role of iron in carcinogenesis: cancer as a ferrotoxic disease, *Cancer Sci.*, 2009, **100**, 9–16.
- 454 L. R. Zacharski, Ferrotoxic disease: the next great public health challenge, *Clin. Chem.*, 2014, **60**, 1362–1364.
- 455 E. Pretorius, N. Vermeulen, J. Bester, B. Lipinski and D. B. Kell, A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy, *Toxicol. Mech. Methods*, 2013, **23**, 352–359.
- 456 E. Pretorius, J. Bester, N. Vermeulen, B. Lipinski, G. S. Gericke and D. B. Kell, Profound morphological changes in the erythrocytes and fibrin networks of patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents, *PLoS One*, 2014, **9**, e85271.
- 457 E. Pretorius, A. C. Swanepoel, A. V. Buys, N. Vermeulen, W. Duim and D. B. Kell, Eryptosis as a marker of Parkinson's disease, *Aging*, 2014, **6**, 788–819.
- 458 E. Pretorius, J. Bester, N. Vermeulen, S. Alummoottil, P. Soma, A. V. Buys and D. B. Kell, Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics, *Cardiovasc. Diabetol.*, 2015, **13**, 30.
- 459 L. Koch, S. Hofer, M. A. Weigand, D. Frommhold, J. Poeschl and P. Ruef, Inhibition of LPS-Induced Activation of Coagulation by p38 MAPK Inhibitor, *ISRN Hematol.*, 2012, **2012**, 762614.
- 460 L. C. Wu, X. Lin and H. Sun, Tanshinone IIA protects rabbits against LPS-induced disseminated intravascular coagulation (DIC), *Acta Pharmacol. Sin.*, 2012, **33**, 1254–1259.
- 461 P. X. Yu, Q. J. Zhou, W. W. Zhu, Y. H. Wu, L. C. Wu, X. Lin, M. H. Chen and B. T. Qiu, Effects of quercetin on LPS-induced disseminated intravascular coagulation (DIC) in rabbits, *Thromb. Res.*, 2013, **131**, e270–e273.
- 462 Z. Wu, J. N. Li, Z. Q. Bai and X. Lin, Antagonism by salvianolic acid B of lipopolysaccharide-induced disseminated intravascular coagulation in rabbits, *Clin. Exp. Pharmacol. Physiol.*, 2014, **41**, 502–508.
- 463 J. Simmons and J. F. Pittet, The coagulopathy of acute sepsis, *Curr. Opin. Anaesthesiol.*, 2015, **28**, 227–236.
- 464 S. Bellary, W. W. Arden, R. W. Schwartz and K. W. Anderson, Effect of lipopolysaccharide, leukocytes, and monoclonal anti-lipid A antibodies on erythrocyte membrane elastance, *Shock*, 1995, **3**, 132–136.
- 465 J. M. B. Pöschl, C. Leray, P. Ruef, J. P. Cazenave and O. Linderkamp, Endotoxin binding to erythrocyte membrane and erythrocyte deformability in human sepsis and *in vitro*, *Crit. Care Med.*, 2003, **31**, 924–928.
- 466 J. Mestres, E. Gregori-Puigjané, S. Valverde and R. V. Solé, The topology of drug-target interaction networks: implicit dependence on drug properties and target families, *Mol. Biosyst.*, 2009, **5**, 1051–1057.
- 467 J. U. Peters, P. Schneider, P. Mattei and M. Kansy, Pharmacological promiscuity: dependence on compound properties and target specificity in a set of recent Roche compounds, *ChemMedChem*, 2009, **4**, 680–686.
- 468 D. A. Price, J. Blagg, L. Jones, N. Greene and T. Wager, Physicochemical drug properties associated with *in vivo* toxicological outcomes: a review, *Expert Opin. Drug Metab. Toxicol.*, 2009, **5**, 921–931.
- 469 P. D. Leeson and J. R. Empfield, Reducing the risk of drug attrition associated with physicochemical properties, *Annu. Rep. Med. Chem.*, 2010, **45**, 393–407.
- 470 F. Saleem, T. C. Bjorndahl, C. L. Ladner, R. Perez-Pineiro, B. N. Ametaj and D. S. Wishart, Lipopolysaccharide induced conversion of recombinant prion protein, *Prion*, 2014, **8**, 221–232.



- 471 A. V. Gyulkhandanyan, A. Mutlu, J. Freedman and V. Leytin, Mitochondrial permeability transition pore (MPTP)-dependent and -independent pathways of mitochondrial membrane depolarization, cell shrinkage and microparticle formation during platelet apoptosis, *Br. J. Haematol.*, 2015, **169**, 142–145.
- 472 M. J. Mooberry and N. S. Key, Microparticle analysis in disorders of hemostasis and thrombosis, *Cytometry, Part A*, 2015, DOI: 10.1002/cyto.a.22647.
- 473 R. M. Thushara, M. Hemshekhar, Basappa, K. Kemparaju, K. S. Rangappa and K. S. Girish, Biologicals, platelet apoptosis and human diseases: an outlook, *Crit. Rev. Oncol. Hematol.*, 2015, **93**, 149–158.
- 474 C. Yang, W. Xiong, Q. Qiu, H. Tahiri, C. Gagnon, G. Liu and P. Hardy, Generation of lymphocytic microparticles and detection of their proapoptotic effect on airway epithelial cells, *J. Visualized Exp.*, 2015, e52651.
- 475 E. Niccolai, G. Emmi, D. Squatrito, E. Silvestri, L. Emmi, A. Amedei and D. Prisco, Microparticles: Bridging the Gap between Autoimmunity and Thrombosis, *Semin. Thromb. Hemostasis*, 2015, **41**, 413–422.
- 476 W. Kolowos, U. S. Gaipal, A. Sheriff, R. E. Voll, P. Heyder, P. Kern, J. R. Kalden and M. Herrmann, Microparticles shed from different antigen-presenting cells display an individual pattern of surface molecules and a distinct potential of allogeneic T-cell activation, *Scand. J. Immunol.*, 2005, **61**, 226–233.
- 477 R. Nieuwland and A. Sturk, Why do cells release vesicles?, *Thromb. Res.*, 2010, **125**(suppl 1), S49–51.
- 478 C. Beyer and D. S. Pisetsky, The role of microparticles in the pathogenesis of rheumatic diseases, *Nat. Rev. Rheumatol.*, 2010, **6**, 21–29.
- 479 S. M. Camus, J. A. De Moraes, P. Bonnin, P. Abbyad, S. Le Jeune, F. Lionnet, L. Loufrani, L. Grimaud, J. C. Lambry, D. Charue, L. Kiger, J. M. Renard, C. Larroque, H. Le Clésiau, A. Tedgui, P. Bruneval, C. Barja-Fidalgo, A. Alexandrou, P. L. Tharaux, C. M. Boulanger and O. P. Blanc-Brude, Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vaso-occlusions in sickle cell disease, *Blood*, 2015, **125**, 3805–3814.
- 480 F. Ciesielski, B. Davis, M. Rittig, B. B. Bonev and P. O'Shea, Receptor-independent interaction of bacterial lipopolysaccharide with lipid and lymphocyte membranes; the role of cholesterol, *PLoS One*, 2012, **7**, e38677.
- 481 M. Lamkanfi and V. M. Dixit, Inflammasomes and their roles in health and disease, *Annu. Rev. Cell Dev. Biol.*, 2012, **28**, 137–161.
- 482 M. Lamkanfi and V. M. Dixit, Mechanisms and functions of inflammasomes, *Cell*, 2014, **157**, 1013–1022.
- 483 P. H. V. Saavedra, D. Demon, H. Van Gorp and M. Lamkanfi, Protective and detrimental roles of inflammasomes in disease, *Semin. Immunopathol.*, 2015, **37**, 313–322.
- 484 Z. Badran, X. Struillou, C. Verner, T. Clee, M. Rakic, M. C. Martinez and A. Soueidan, Periodontitis as a risk factor for systemic disease: are microparticles the missing link?, *Med. Hypotheses*, 2015, **84**, 555–556.
- 485 A. Berezin, A. Zulli, S. Kerrigan, D. Petrovic and P. Kruzliak, Predictive role of circulating endothelial-derived microparticles in cardiovascular diseases, *Clin. Biochem.*, 2015, **42**, 562–568.
- 486 J. Rodríguez-Carrio, M. Alperi-López, P. Lopez, S. Alonso-Castro, S. R. Carro-Esteban, F. J. Ballina-García and A. Suarez, Altered profile of circulating microparticles in rheumatoid arthritis patients, *Clin. Sci.*, 2015, **128**, 437–448.
- 487 M. A. E. K. Salem, A. A. M. Adly, E. A. R. Ismail, Y. W. Darwish and H. A. Kamel, Platelets microparticles as a link between micro- and macro-angiopathy in young patients with type 1 diabetes, *Platelets*, 2015, 1–7.
- 488 J. M. Alam and M. Yamazaki, Spontaneous insertion of lipopolysaccharide into lipid membranes from aqueous solution, *Chem. Phys. Lipids*, 2011, **164**, 166–174.
- 489 F. Ciesielski, D. C. Griffin, M. Rittig, I. Moriyon and B. B. Bonev, Interactions of lipopolysaccharide with lipid membranes, raft models – a solid state NMR study, *Biochim. Biophys. Acta*, 2013, **1828**, 1731–1742.
- 490 K. Nomura, M. Maeda, K. Sugase and S. Kusumoto, Lipopolysaccharide induces raft domain expansion in membrane composed of a phospholipid-cholesterol-sphingomyelin ternary system, *Innate Immun.*, 2011, **17**, 256–268.
- 491 R. J. Berckmans, R. Nieuwland, P. P. Tak, A. N. Böing, F. P. H. T. M. Romijn, M. C. Kraan, F. C. Breedveld, C. E. Hack and A. Sturk, Cell-derived microparticles in synovial fluid from inflamed arthritic joints support coagulation exclusively via a factor VII-dependent mechanism, *Arthritis Rheum.*, 2002, **46**, 2857–2866.
- 492 E. F. Midura, P. L. Jernigan, J. W. Kuethe, L. A. Friend, R. Veile, A. T. Makley, C. C. Caldwell and M. D. Goodman, Microparticles impact coagulation after traumatic brain injury, *J. Surg. Res.*, 2015, **197**, 25–31.
- 493 K. Labbé and M. Saleh, Cell death in the host response to infection, *Cell Death Differ.*, 2008, **15**, 1339–1349.
- 494 G. Yeretssian, K. Labbé and M. Saleh, Molecular regulation of inflammation and cell death, *Cytokine*, 2008, **43**, 380–390.
- 495 S. D. Kobayashi, K. M. Rigby and F. R. DeLeo, Bacteria-induced Host Cell Death, *Bact. Pathog.*, 2012, 317–362.
- 496 E. Clark, C. Hoare, J. Tanianis-Hughes, G. L. Carlson and G. Warhurst, Interferon gamma induces translocation of commensal *Escherichia coli* across gut epithelial cells via a lipid raft-mediated process, *Gastroenterology*, 2005, **128**, 1258–1267.
- 497 A. T. Blikslager, A. J. Moeser, J. L. Gookin, S. L. Jones and J. Odle, Restoration of barrier function in injured intestinal mucosa, *Physiol. Rev.*, 2007, **87**, 545–564.
- 498 A. Fasano, Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer, *Physiol. Rev.*, 2011, **91**, 151–175.
- 499 T. H. Frazier, J. K. DiBaise and C. J. McClain, Gut microbiota, intestinal permeability, obesity-induced inflammation, and liver injury, *JPEN, J. Parenter. Enteral Nutr.*, 2011, **35**, 14S–20S.
- 500 L. J. John, M. Fromm and J. D. Schulzke, Epithelial barriers in intestinal inflammation, *Antioxid. Redox Signaling*, 2011, **15**, 1255–1270.





- 501 Y. Ilan, Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis, *World J. Gastroenterol.*, 2012, **18**, 2609–2618.
- 502 T. F. S. Teixeira, M. C. Collado, C. L. L. F. Ferreira, J. Bressan and C. G. di Peluzio Mdo, Potential mechanisms for the emerging link between obesity and increased intestinal permeability, *Nutr. Res.*, 2012, **32**, 637–647.
- 503 T. F. S. Teixeira, N. C. S. Souza, P. G. Chiarello, S. C. C. Franceschini, J. Bressan, C. L. L. F. Ferreira and C. G. di Peluzio Mdo, Intestinal permeability parameters in obese patients are correlated with metabolic syndrome risk factors, *Clin. Nutr.*, 2012, **31**, 735–740.
- 504 N. Ijssennagger, C. Belzer, G. J. Hooiveld, J. Dekker, S. W. van Mil, M. Muller, M. Kleerebezem and R. van der Meer, Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 10038–10043.
- 505 X. Li and M. A. Atkinson, The role for gut permeability in the pathogenesis of type 1 diabetes – a solid or leaky concept?, *Pediatr. Diabetes*, 2015, DOI: 10.1111/pedi.12305.
- 506 P. Yu and C. M. Martin, Increased gut permeability and bacterial translocation in *Pseudomonas pneumonia*-induced sepsis, *Crit. Care Med.*, 2000, **28**, 2573–2577.
- 507 J. M. Brenchley, D. A. Price and D. C. Douek, HIV disease: fallout from a mucosal catastrophe?, *Nat. Immunol.*, 2006, **7**, 235–239.
- 508 P. Vassallo and R. G. Trohman, Prescribing amiodarone: an evidence-based review of clinical indications, *JAMA, J. Am. Med. Assoc.*, 2007, **298**, 1312–1322.
- 509 W. Jiang, M. M. Lederman, P. Hunt, S. F. Sieg, K. Haley, B. Rodriguez, A. Landay, J. Martin, E. Sinclair, A. I. Asher, S. G. Deeks, D. C. Douek and J. M. Brenchley, Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection, *J. Infect. Dis.*, 2009, **199**, 1177–1185.
- 510 D. Kevans, W. Turpin, K. Madsen, J. Meddings, K. Shestopaloff, W. Xu, G. Moreno-Hagelsieb, A. Griffiths, M. S. Silverberg, A. Paterson, K. Croitoru and G. E. M. Project, Determinants of intestinal permeability in healthy first-degree relatives of individuals with Crohn's disease, *Inflammatory Bowel Dis.*, 2015, **21**, 879–887.
- 511 X. Han, M. P. Fink, R. Yang and R. L. Delude, Increased iNOS activity is essential for intestinal epithelial tight junction dysfunction in endotoxemic mice, *Shock*, 2004, **21**, 261–270.
- 512 M. Schietroma, F. Carlei, S. Cappelli and G. Amicucci, Intestinal permeability and systemic endotoxemia after laparotomic or laparoscopic cholecystectomy, *Ann. Surg.*, 2006, **243**, 359–363.
- 513 J. Zhaowei, M. J. D'Souza and C. W. Oettinger, Reversal of LPS induced endothelial cell TNF synthesis and increased permeability with microencapsulated antisense oligomers to NF-kappaB, *J. Microencapsulation*, 2007, **24**, 596–607.
- 514 C. T. Capaldo and A. Nusrat, Cytokine regulation of tight junctions, *Biochim. Biophys. Acta*, 2009, **1788**, 864–871.
- 515 M. Schietroma, B. Pessia, F. Carlei, E. M. Cecilia and G. Amicucci, Intestinal permeability, systemic endotoxemia, and bacterial translocation after open or laparoscopic resection for colon cancer: a prospective randomized study, *Int. J. Colorectal Dis.*, 2013, **28**, 1651–1660.
- 516 Z. Ruan, S. Liu, Y. Zhou, S. Mi, G. Liu, X. Wu, K. Yao, H. Assaad, Z. Deng, Y. Hou, G. Wu and Y. Yin, Chlorogenic acid decreases intestinal permeability and increases expression of intestinal tight junction proteins in weaned rats challenged with LPS, *PLoS One*, 2014, **9**, e97815.
- 517 C. Funke, S. A. Schneider, D. Berg and D. B. Kell, Genetics and iron in the systems biology of Parkinson's disease and some related disorders, *Neurochem. Int.*, 2013, **62**, 637–652.
- 518 N. Singh, The role of iron in prion disease and other neurodegenerative diseases, *PLoS Pathog.*, 2014, **10**, e1004335.
- 519 G. G. Tedeschi, D. Amici and M. Paparelli, Incorporation of nucleosides and amino-acids in human erythrocyte suspensions: possible relation with a diffuse infection of mycoplasmas or bacteria in the L form, *Nature*, 1969, **222**, 1285–1286.
- 520 G. G. Tedeschi, A. Bondi, M. Paparelli and G. Sprovieri, Electron microscopical evidence of the evolution of corynebacteria-like microorganisms within human erythrocytes, *Experientia*, 1978, **34**, 458–460.
- 521 R. W. McLaughlin, H. Vali, P. C. Lau, R. G. E. Palfree, A. De Ciccio, M. Sirois, D. Ahmad, R. Villemur, M. Desrosiers and E. C. S. Chan, Are there naturally occurring pleomorphic bacteria in the blood of healthy humans?, *J. Clin. Microbiol.*, 2002, **40**, 4771–4775.
- 522 O. H. Munz, S. Sela, B. S. Baker, C. E. M. Griffiths, A. V. Powles and L. Fry, Evidence for the presence of bacteria in the blood of psoriasis patients, *Arch. Dermatol. Res.*, 2010, **302**, 495–498.
- 523 P. A. Leppäluoto, Bacterial vaginosis: what is physiological in vaginal bacteriology? An update and opinion, *Acta Obstet. Gynecol. Scand.*, 2011, **90**, 1302–1306.
- 524 R. W. Hyman, M. Fukushima, H. Jiang, E. Fung, L. Rand, B. Johnson, K. C. Vo, A. B. Caughey, J. F. Hilton, R. W. Davis and L. C. Giudice, Diversity of the vaginal microbiome correlates with preterm birth, *Reprod. Sci.*, 2014, **21**, 32–40.
- 525 D. B. DiGiulio, B. J. Callahan, P. J. McMurdie, E. K. Costello, D. J. Lyell, A. Robaczewska, C. L. Sun, D. S. Goltzman, R. J. Wong, G. Shaw, D. K. Stevenson, S. P. Holmes and D. A. Relman, Temporal and spatial variation of the human microbiota during pregnancy, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 11060–11065.
- 526 K. Aagaard, J. Ma, K. M. Antony, R. Ganu, J. Petrosino and J. Versalovic, The placenta harbors a unique microbiome, *Sci. Transl. Med.*, 2014, **6**, 237ra265.
- 527 R. Amarasekara, R. W. Jayasekara, H. Senanayake and V. H. Dissanayake, Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia, *J. Obstet. Gynaecol. Res.*, 2015, **41**, 662–669.



- 528 B. Cao, M. J. Stout, I. Lee and I. U. Mysorekar, Placental Microbiome and Its Role in Preterm Birth, *Neoreviews*, 2014, **15**, e537–e545.
- 529 V. Blanc, F. O Valle, E. Pozo, A. Puertas, R. Leon and F. Mesa, Oral bacteria in placental tissues: increased molecular detection in pregnant periodontitis patients, *Oral Dis.*, 2015, DOI: 10.1111/odi.12364.
- 530 J. Zheng, X. Xiao, Q. Zhang, L. Mao, M. Yu and J. Xu, The Placental Microbiome Varies in Association with Low Birth Weight in Full-Term Neonates, *Nutrients*, 2015, **7**, 6924–6937.
- 531 C. Gardella, D. E. Riley, J. Hitti, K. Agnew, J. N. Krieger and D. Eschenbach, Identification and sequencing of bacterial rDNAs in culture-negative amniotic fluid from women in premature labor, *Am. J. Perinatol.*, 2004, **21**, 319–323.
- 532 D. B. DiGiulio, R. Romero, H. P. Amogan, J. P. Kusanovic, E. M. Bik, F. Gotsch, C. J. Kim, O. Erez, S. Edwin and D. A. Relman, Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation, *PLoS One*, 2008, **3**, e3056.
- 533 D. B. DiGiulio, M. T. Gervasi, R. Romero, E. Vaisbuch, S. Mazaki-Tovi, J. P. Kusanovic, K. S. Seok, R. Gomez, P. Mittal, F. Gotsch, T. Chaiworapongsa, E. Oyarzun, C. J. Kim and D. A. Relman, Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses, *J. Perinat. Med.*, 2010, **38**, 495–502.
- 534 D. B. DiGiulio, M. Gervasi, R. Romero, S. Mazaki-Tovi, E. Vaisbuch, J. P. Kusanovic, K. S. Seok, R. Gomez, P. Mittal, F. Gotsch, T. Chaiworapongsa, E. Oyarzun, C. J. Kim and D. A. Relman, Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods, *J. Perinat. Med.*, 2010, **38**, 503–513.
- 535 D. B. DiGiulio, R. Romero, J. P. Kusanovic, R. Gomez, C. J. Kim, K. S. Seok, F. Gotsch, S. Mazaki-Tovi, E. Vaisbuch, K. Sanders, E. M. Bik, T. Chaiworapongsa, E. Oyarzun and D. A. Relman, Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes, *Am. J. Reprod. Immunol.*, 2010, **64**, 38–57.
- 536 D. B. DiGiulio, Diversity of microbes in amniotic fluid, *Semin. Fetal Neonatal. Med.*, 2012, **17**, 2–11.
- 537 R. Romero, J. Miranda, T. Chaiworapongsa, P. Chaemsaitong, F. Gotsch, Z. Dong, A. I. Ahmed, B. H. Yoon, S. S. Hassan, C. J. Kim, S. J. Korzeniewski and L. Yeo, A novel molecular microbiologic technique for the rapid diagnosis of microbial invasion of the amniotic cavity and intra-amniotic infection in preterm labor with intact membranes, *Am. J. Reprod. Immunol.*, 2014, **71**, 330–358.
- 538 L. F. Stinson, D. J. Ireland, M. W. Kemp, M. S. Payne, S. J. Stock, J. P. Newnham and J. A. Keelan, Effects of cytokine-suppressive anti-inflammatory drugs on inflammatory activation in *ex vivo* human and ovine fetal membranes, *Reproduction*, 2014, **147**, 313–320.
- 539 P. Y. Ng, D. J. Ireland and J. A. Keelan, Drugs to block cytokine signaling for the prevention and treatment of inflammation-induced preterm birth, *Front. Immunol.*, 2015, **6**, 166.
- 540 R. Romero, J. Miranda, J. P. Kusanovic, T. Chaiworapongsa, P. Chaemsaitong, A. Martinez, F. Gotsch, Z. Dong, A. I. Ahmed, M. Shaman, K. Lannaman, B. H. Yoon, S. S. Hassan, C. J. Kim, S. J. Korzeniewski, L. Yeo and Y. M. Kim, Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques, *J. Perinat. Med.*, 2015, **43**, 19–36.
- 541 T. Nakatsuji, H. I. Chiang, S. B. Jiang, H. Nagarajan, K. Zengler and R. L. Gallo, The microbiome extends to subepidermal compartments of normal skin, *Nat. Commun.*, 2013, **4**, 1431.
- 542 T. Trotta, C. Porro, R. Calvello and M. A. Panaro, Biological role of Toll-like receptor-4 in the brain, *J. Neuroimmunol.*, 2014, **268**, 1–12.
- 543 A. Ebringer, T. Rashid and C. Wilson, Rheumatoid arthritis, Proteus, anti-CCP antibodies and Karl Popper, *Autoimmun. Rev.*, 2010, **9**, 216–223.
- 544 A. Ebringer, *Rheumatoid Arthritis and Proteus*, Springer, London, 2012.
- 545 A. Ebringer and T. Rashid, Rheumatoid arthritis is caused by a *Proteus* urinary tract infection, *APMIS*, 2014, **122**, 363–368.
- 546 X. Zhang, D. Zhang, H. Jia, Q. Feng, D. Wang, D. Liang, X. Wu, J. Li, L. Tang, Y. Li, Z. Lan, B. Chen, Y. Li, H. Zhong, H. Xie, Z. Jie, W. Chen, S. Tang, X. Xu, X. Wang, X. Cai, S. Liu, Y. Xia, J. Li, X. Qiao, J. Y. Al-Aama, H. Chen, L. Wang, Q. J. Wu, F. Zhang, W. Zheng, Y. Li, M. Zhang, G. Luo, W. Xue, L. Xiao, J. Li, W. Chen, X. Xu, Y. Yin, H. Yang, J. Wang, K. Kristiansen, L. Liu, T. Li, Q. Huang, Y. Li and J. Wang, The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment, *Nat. Med.*, 2015, **21**, 895–905.
- 547 D. W. Dresser and A. M. Popham, Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide, *Nature*, 1976, **264**, 552–554.
- 548 S. Izui, R. A. Eisenberg and F. J. Dixon, IgM rheumatoid factors in mice injected with bacterial lipopolysaccharides, *J. Immunol.*, 1979, **122**, 2096–2102.
- 549 P. J. Meredith, J. A. Kristie and R. L. Walford, Aging increases expression of LPS-induced autoantibody-secreting B cells, *J. Immunol.*, 1979, **123**, 87–91.
- 550 M. Fujiwara, A. Kariyone and M. Kimura, Tolerance inducibility and the elicitation of autoantibodies by LPS in aged NZB mice, *J. Clin. Lab. Immunol.*, 1980, **3**, 185–188.
- 551 R. Dziarski, Preferential induction of autoantibody secretion in polyclonal activation by peptidoglycan and lipopolysaccharide. I. *In vitro* studies, *J. Immunol.*, 1982, **128**, 1018–1025.
- 552 R. Dziarski, Preferential induction of autoantibody secretion in polyclonal activation by peptidoglycan and lipopolysaccharide. II. *In vivo* studies, *J. Immunol.*, 1982, **128**, 1026–1030.
- 553 R. Dziarski, Comparison of *in vitro* and *in vivo* mitogenic and polyclonal antibody and autoantibody responses to



- peptidoglycan, LPS, protein A, PWM, PHA and Con A in normal and autoimmune mice, *J. Clin. Lab. Immunol.*, 1985, **16**, 93–109.
- 554 L. M. Hang, M. T. Aguado, F. J. Dixon and A. N. Theofilopoulos, Induction of severe autoimmune disease in normal mice by simultaneous action of multiple immunostimulators, *J. Exp. Med.*, 1985, **161**, 423–428.
- 555 D. N. Posnett and J. Edinger, When do microbes stimulate rheumatoid factor?, *J. Exp. Med.*, 1997, **185**, 1721–1723.
- 556 M. Satoh, V. M. Shaheen, M. Shaw, H. B. Richards and W. H. Reeves, Role of bacterial lipopolysaccharide (LPS) in the induction of Lupus autoantibodies by pristane, *Arthritis Rheum.*, 1998, **41**, S178–S178.
- 557 R. D. Yammani, M. A. Leyva, R. N. Jennings and K. M. Haas, C4 Deficiency is a predisposing factor for *Streptococcus pneumoniae*-induced autoantibody production, *J. Immunol.*, 2014, **193**, 5434–5443.
- 558 V. Petrušić, N. Todorović, I. Živković, R. Dimitrijević, L. Muhandes, I. Rajnpreht and L. Dimitrijević, Autoantibody response and pregnancy-related pathology induced by combined LPS and tetanus toxoid hyperimmunization in BALB/c and C57BL/6 mice, *Autoimmunity*, 2015, **48**, 87–99.
- 559 S. M. Yentis, N. Soni and P. G. Riches, *In vitro* effects of HA-1A (Centoxin) on cytokine production in whole blood from intensive care unit patients, *Br. J. Anaesth.*, 1994, **73**, 805–811.
- 560 L. Marks, The birth pangs of monoclonal antibody therapeutics: the failure and legacy of Centoxin, *MABs*, 2012, **4**, 403–412.
- 561 P. J. Albert, A. D. Proal and T. G. Marshall, Vitamin D: the alternative hypothesis, *Autoimmun. Rev.*, 2009, **8**, 639–644.
- 562 G. P. Blaney, P. J. Albert and A. D. Proal, Vitamin D metabolites as clinical markers in autoimmune and chronic disease, *Ann. N. Y. Acad. Sci.*, 2009, **1173**, 384–390.
- 563 A. D. Proal, P. J. Albert and T. Marshall, Autoimmune disease in the era of the metagenome, *Autoimmun. Rev.*, 2009, **8**, 677–681.
- 564 A. D. Proal, P. J. Albert, G. P. Blaney, I. A. Lindseth, C. Benediktsson and T. G. Marshall, Immunostimulation in the era of the metagenome, *Cell. Mol. Immunol.*, 2011, **8**, 213–225.
- 565 A. D. Proal, P. J. Albert, T. G. Marshall, G. P. Blaney and I. A. Lindseth, Immunostimulation in the treatment for chronic fatigue syndrome/myalgic encephalomyelitis, *Immunol. Res.*, 2013, **56**, 398–412.
- 566 M. Maes, I. Mihaylova and J. C. Leunis, Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): indication for the involvement of Gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut-intestinal permeability, *J. Affective Disord.*, 2007, **99**, 237–240.
- 567 M. Maes, M. Kubera and J. C. Leunis, The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression, *Neuroendocrinol. Lett.*, 2008, **29**, 117–124.
- 568 M. Maes, Leaky gut in chronic fatigue syndrome: a review, *Activitas Nervosa Superior Rediviva*, 2009, **51**, 21–28.
- 569 M. Maes, M. Kubera, J. C. Leunis and M. Berk, Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut, *J. Affective Disord.*, 2012, **141**, 55–62.
- 570 M. Maes, F. N. M. Twisk, M. Kubera, K. Ringel, J. C. Leunis and M. Geffard, Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome, *J. Affective Disord.*, 2012, **136**, 909–917.
- 571 M. Lyte, The role of catecholamines in Gram-negative sepsis, *Med. Hypotheses*, 1992, **37**, 255–258.
- 572 M. Lyte and S. Ernst, Catecholamine-induced growth of Gram-negative bacteria, *Life Sci.*, 1992, **50**, 203–212.
- 573 M. Lyte, The role of microbial endocrinology in infectious disease, *J. Endocrinol.*, 1993, **137**, 343–345.
- 574 P. P. E. Freestone and M. Lyte, Microbial endocrinology: experimental design issues in the study of interkingdom signalling in infectious disease, *Adv. Appl. Microbiol.*, 2008, **64**, 75–105.
- 575 M. Lyte, Microbial endocrinology and infectious disease in the 21st century, *Trends Microbiol.*, 2004, **12**, 14–20.
- 576 P. P. E. Freestone, S. M. Sandrini, R. D. Haigh and M. Lyte, Microbial endocrinology: how stress influences susceptibility to infection, *Trends Microbiol.*, 2008, **16**, 55–64.
- 577 M. Lyte, Microbial endocrinology: host-microbiota neuroendocrine interactions influencing brain and behavior, *Gut Microbes*, 2014, **5**, 381–389.
- 578 J. D. Galley, M. C. Nelson, Z. Yu, S. E. Dowd, J. Walter, P. S. Kumar, M. Lyte and M. T. Bailey, Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota, *BMC Microbiol.*, 2014, **14**, 189.
- 579 M. Lyte, The effect of stress on microbial growth, *Anim. Health Res. Rev.*, 2014, **15**, 172–174.
- 580 S. W. Brown, R. T. Meyers, K. M. Brennan, J. M. Rumble, N. Narasimhachari, E. F. Perozzi, J. J. Ryan, J. K. Stewart and K. Fischer-Stenger, Catecholamines in a macrophage cell line, *J. Neuroimmunol.*, 2003, **135**, 47–55.
- 581 K. L. Engler, M. L. Rudd, J. J. Ryan, J. K. Stewart and K. Fischer-Stenger, Autocrine actions of macrophage-derived catecholamines on interleukin-1 beta, *J. Neuroimmunol.*, 2005, **160**, 87–91.
- 582 M. A. Flierl, D. Rittirsch, B. A. Nadeau, A. J. Chen, J. V. Sarma, F. S. Zetoune, S. R. McGuire, R. P. List, D. E. Day, L. M. Hoesel, H. Gao, N. Van Rooijen, M. S. Huber-Lang, R. R. Neubig and P. A. Ward, Phagocyte-derived catecholamines enhance acute inflammatory injury, *Nature*, 2007, **449**, 721–725.
- 583 M. A. Flierl, D. Rittirsch, B. A. Nadeau, J. V. Sarma, D. E. Day, A. B. Lentsch, M. S. Huber-Lang and P. A. Ward, Upregulation of phagocyte-derived catecholamines augments the acute inflammatory response, *PLoS One*, 2009, **4**, e4414.
- 584 P. P. Freestone, M. Lyte, C. P. Neal, A. F. Maggs, R. D. Haigh and P. H. Williams, The mammalian neuroendocrine



- hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin, *J. Bacteriol.*, 2000, **182**, 6091–6098.
- 585 P. P. E. Freestone, R. D. Haigh, P. H. Williams and M. Lyte, Involvement of enterobactin in norepinephrine-mediated iron supply from transferrin to enterohaemorrhagic *Escherichia coli*, *FEMS Microbiol. Lett.*, 2003, **222**, 39–43.
- 586 S. M. Sandrini, R. Shergill, J. Woodward, R. Muralikuttan, R. D. Haigh, M. Lyte and P. P. Freestone, Elucidation of the mechanism by which catecholamine stress hormones liberate iron from the innate immune defense proteins transferrin and lactoferrin, *J. Bacteriol.*, 2010, **192**, 587–594.
- 587 R. J. Abergel, M. C. Clifton, J. C. Pizarro, J. A. Warner, D. K. Shuh, R. K. Strong and K. N. Raymond, The siderocalin/enterobactin interaction: a link between mammalian immunity and bacterial iron transport, *J. Am. Chem. Soc.*, 2008, **130**, 11524–11534.
- 588 D. W. Reid, G. J. Anderson and I. L. Lamont, Role of lung iron in determining the bacterial and host struggle in cystic fibrosis, *Am. J. Physiol.: Lung Cell. Mol. Physiol.*, 2009, **297**, L795–802.
- 589 M. Nairz, A. Schroll, T. Sonnweber and G. Weiss, The struggle for iron – a metal at the host–pathogen interface, *Cell. Microbiol.*, 2010, **12**, 1691–1702.
- 590 A. K. Sia, B. E. Allred and K. N. Raymond, Siderocalins: Siderophore binding proteins evolved for primary pathogen host defense, *Curr. Opin. Chem. Biol.*, 2013, **17**, 150–157.
- 591 N. Leon-Sicairos, R. Reyes-Cortes, A. M. Guadrón-Llanos, J. Madueña-Molina, C. Leon-Sicairos and A. Canizalez-Román, Strategies of Intracellular Pathogens for Obtaining Iron from the Environment, *BioMed Res. Int.*, 2015, **2015**, 476534.
- 592 F. Meziani, X. Delabranche, P. Asfar and F. Toti, Bench-to-bedside review: circulating microparticles – a new player in sepsis?, *Crit. Care*, 2010, **14**, 236.
- 593 V. L. Reid and N. R. Webster, Role of microparticles in sepsis, *Br. J. Anaesth.*, 2012, **109**, 503–513.
- 594 D. Annane, E. Bellissant and J. M. Cavillon, Septic shock, *Lancet*, 2005, **365**, 63–78.
- 595 R. S. Parker and G. Clermont, Systems engineering medicine: engineering the inflammation response to infectious and traumatic challenges, *J. R. Soc., Interface*, 2010, **7**, 989–1013.
- 596 M. P. Fink and H. S. Warren, Strategies to improve drug development for sepsis, *Nat. Rev. Drug Discovery*, 2014, **13**, 741–758.
- 597 J. C. Marshall, Why have clinical trials in sepsis failed?, *Trends Mol. Med.*, 2014, **20**, 195–203.
- 598 J. B. German, L. A. Gillies, J. T. Smilowitz, A. M. Zivkovic and S. M. Watkins, Lipidomics and lipid profiling in metabolomics, *Curr. Opin. Lipidol.*, 2007, **18**, 66–71.
- 599 M. J. O. Wakelam, T. R. Pettitt and A. D. Postle, Lipidomic analysis of signaling pathways, *Methods Enzymol.*, 2007, **432**, 233–246.
- 600 M. Orešič, V. A. Hanninen and A. Vidal-Puig, Lipidomics: a new window to biomedical frontiers, *Trends Biotechnol.*, 2008, **26**, 647–652.
- 601 M. Sud, E. Fahy, D. Cotter, E. A. Dennis and S. Subramaniam, LIPID MAPS-Nature Lipidomics Gateway: An Online Resource for Students and Educators Interested in Lipids, *J. Chem. Educ.*, 2012, **89**, 291–292.
- 602 J. M. Foster, P. Moreno, A. Fabregat, H. Hermjakob, C. Steinbeck, R. Apweiler, M. J. O. Wakelam and J. A. Vizcaino, LipidHome: a database of theoretical lipids optimized for high throughput mass spectrometry lipidomics, *PLoS One*, 2013, **8**, e61951.
- 603 M. S. Köberlin, B. Snijder, L. X. Heinz, C. L. Baumann, A. Fauster, G. I. Vladimer, A. C. Gavin and G. Superti-Furga, A Conserved Circular Network of Coregulated Lipids Modulates Innate Immune Responses, *Cell*, 2015, **162**, 170–183.
- 604 E. J. Ziegler, C. J. Fisher Jr., C. L. Sprung, R. C. Straube, J. C. Sadoff, G. E. Foulke, C. H. Wortel, M. P. Fink, R. P. Dellinger and N. N. Teng, *et al.*, Treatment of Gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group, *N. Engl. J. Med.*, 1991, **324**, 429–436.
- 605 J. P. A. Ioannidis, Why most published research findings are false, *PLoS Med.*, 2005, **2**, e124.
- 606 D. Broadhurst and D. B. Kell, Statistical strategies for avoiding false discoveries in metabolomics and related experiments, *Metabolomics*, 2006, **2**, 171–196.
- 607 B. Derkx, J. Wittes and R. McCloskey, Randomized, placebo-controlled trial of HA-1A, a human monoclonal antibody to endotoxin, in children with meningococcal septic shock. European Pediatric Meningococcal Septic Shock Trial Study Group, *Clin. Infect. Dis.*, 1999, **28**, 770–777.
- 608 J. D. Baumgartner and M. P. Glauser, Immunotherapy of endotoxemia and septicemia, *Immunobiology*, 1993, **187**, 464–477.
- 609 E. J. Helmerhorst, J. J. Maaskant and B. J. Appelmelk, Anti-lipid A monoclonal antibody centoxin (HA-1A) binds to a wide variety of hydrophobic ligands, *Infect. Immun.*, 1998, **66**, 870–873.
- 610 S. Ekins, Predicting undesirable drug interactions with promiscuous proteins *in silico*, *Drug Discovery Today*, 2004, **9**, 276–285.
- 611 P. D. Leeson and B. Springthorpe, The influence of drug-like concepts on decision-making in medicinal chemistry, *Nat. Rev. Drug Discovery*, 2007, **6**, 881–890.
- 612 P. D. Leeson and S. A. St-Gallay, The influence of the ‘organizational factor’ on compound quality in drug discovery, *Nat. Rev. Drug Discovery*, 2011, **10**, 749–765.
- 613 N. S. Tan, M. L. Ng, Y. H. Yau, P. K. W. Chong, B. Ho and J. L. Ding, Definition of endotoxin binding sites in horseshoe crab factor C recombinant sushi proteins and neutralization of endotoxin by sushi peptides, *FASEB J.*, 2000, **14**, 1801–1813.
- 614 N. S. Tan, B. Ho and J. L. Ding, High-affinity LPS binding domain(s) in recombinant factor C of a horseshoe crab neutralizes LPS-induced lethality, *FASEB J.*, 2000, **14**, 859–870.
- 615 J. L. Ding, P. Li and B. Ho, The Sushi peptides: structural characterization and mode of action against Gram-negative bacteria, *Cell. Mol. Life Sci.*, 2008, **65**, 1202–1219.



- 616 Y. H. Yau, B. Ho, N. S. Tan, M. L. Ng and J. L. Ding, High therapeutic index of factor C Sushi peptides: potent antimicrobials against *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.*, 2001, **45**, 2820–2825.
- 617 S. Leptihn, J. Y. Har, J. Chen, B. Ho, T. Wohland and J. L. Ding, Single molecule resolution of the antimicrobial action of quantum dot-labeled sushi peptide on live bacteria, *BMC Biol.*, 2009, **7**, 22.
- 618 S. Leptihn, L. Guo, V. Frecer, B. Ho, J. L. Ding and T. Wohland, One step at a time: action mechanism of Sushi 1 antimicrobial peptide and derived molecules, *Virulence*, 2010, **1**, 42–44.
- 619 P. Li, M. Sun, T. Wohland, B. Ho and J. L. Ding, The molecular mechanism of interaction between sushi peptide and *Pseudomonas* endotoxin, *Cell. Mol. Immunol.*, 2006, **3**, 21–28.
- 620 P. Li, M. Sun, T. Wohland, D. Yang, B. Ho and J. L. Ding, Molecular mechanisms that govern the specificity of Sushi peptides for Gram-negative bacterial membrane lipids, *Biochemistry*, 2006, **45**, 10554–10562.
- 621 P. Li, M. Sun, B. Ho and J. L. Ding, The specificity of Sushi peptides for endotoxin and anionic phospholipids: potential application of POPG as an adjuvant for anti-LPS strategies, *Biochem. Soc. Trans.*, 2006, **34**, 270–272.
- 622 V. Frecer, B. Ho and J. L. Ding, *De novo* design of potent antimicrobial peptides, *Antimicrob. Agents Chemother.*, 2004, **48**, 3349–3357.
- 623 S. Bhattacharjya, *De novo* designed lipopolysaccharide binding peptides: structure based development of antiendotoxic and antimicrobial drugs, *Curr. Med. Chem.*, 2010, **17**, 3080–3093.
- 624 A. Skerra, Anticalins as alternative binding proteins for therapeutic use, *Curr. Opin. Mol. Ther.*, 2007, **9**, 336–344.
- 625 M. Gebauer and A. Skerra, Anticalins small engineered binding proteins based on the lipocalin scaffold, *Methods Enzymol.*, 2012, **503**, 157–188.
- 626 E. Eggenstein, A. Eichinger, H. J. Kim and A. Skerra, Structure-guided engineering of Anticalins with improved binding behavior and biochemical characteristics for application in radio-immuno imaging and/or therapy, *J. Struct. Biol.*, 2014, **185**, 203–214.
- 627 A. Richter, E. Eggenstein and A. Skerra, Anticalins: exploiting a non-Ig scaffold with hypervariable loops for the engineering of binding proteins, *FEBS Lett.*, 2014, **588**, 213–218.
- 628 A. Schiefner and A. Skerra, The menagerie of human lipocalins: a natural protein scaffold for molecular recognition of physiological compounds, *Acc. Chem. Res.*, 2015, **48**, 976–985.
- 629 E. Hailman, H. S. Lichenstein, M. M. Wurfel, D. S. Miller, D. A. Johnson, M. Kelley, L. A. Busse, M. M. Zukowski and S. D. Wright, Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14, *J. Exp. Med.*, 1994, **179**, 269–277.
- 630 S. Lengacher, C. V. Jongeneel, D. Le Roy, J. D. Lee, V. Kravchenko, R. J. Ulevitch, M. P. Glauser and D. Heumann, Reactivity of murine and human recombinant LPS-binding protein (LBP) within LPS and gram negative bacteria, *J. Inflammation*, 1995, **47**, 165–172.
- 631 P. Li, B. Ho and J. L. Ding, Recombinant factor C competes against LBP to bind lipopolysaccharide and neutralizes the endotoxicity, *J. Endotoxin Res.*, 2007, **13**, 150–157.
- 632 E. D. Weinberg and J. Miklossy, Iron withholding: a defense against disease, *J. Alzheimer's Dis.*, 2008, **13**, 451–463.
- 633 G. Srinivasan, J. D. Aitken, B. Zhang, F. A. Carvalho, B. Chassaing, R. Shashidharamurthy, N. Borregaard, D. P. Jones, A. T. Gewirtz and M. Vijay-Kumar, Lipocalin 2 deficiency dysregulates iron homeostasis and exacerbates endotoxin-induced sepsis, *J. Immunol.*, 2012, **189**, 1911–1919.
- 634 C. Lehmann, N. Sharawi, N. Al-Banna, N. Corbett, J. W. Kuethe and C. C. Caldwell, Novel approaches to the development of anti-sepsis drugs, *Expert Opin. Drug Discovery*, 2014, **9**, 523–531.
- 635 G. Luo, B. Spellberg, T. Gebremariam, H. Lee, Y. Q. Xiong, S. W. French, A. Bayer and A. S. Ibrahim, Combination therapy with iron chelation and vancomycin in treating murine staphylococemia, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2014, **33**, 845–851.
- 636 C. Zeng, Q. Chen, K. Zhang, Q. Chen, S. Song and X. Fang, Hepatic Hecidin Protects against Polymicrobial Sepsis in Mice by Regulating Host Iron Status, *Anesthesiology*, 2015, **122**, 374–386.
- 637 M. B. Zimmermann, C. Chassard, F. Rohner, E. K. N'Goran, C. Nindjin, A. Dostal, J. Utzinger, H. Ghattas, C. Lacroix and R. F. Hurrell, The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire, *Am. J. Clin. Nutr.*, 2010, **92**, 1406–1415.
- 638 T. Jaeggi, G. A. Kortman, D. Moretti, C. Chassard, P. Holding, A. Dostal, J. Boekhorst, H. M. Timmerman, D. W. Swinkels, H. Tjalsma, J. Njenga, A. Mwangi, J. Kvalsvig, C. Lacroix and M. B. Zimmermann, Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants, *Gut*, 2015, **64**, 731–742.
- 639 N. R. Perron and J. L. Brumaghim, A review of the antioxidant mechanisms of polyphenol compounds related to iron binding, *Cell Biochem. Biophys.*, 2009, **53**, 75–100.
- 640 V. Chobot and F. Hadacek, Iron and its complexation by phenolic cellular metabolites: from oxidative stress to chemical weapons, *Plant Signaling Behav.*, 2010, **5**, 4–8.
- 641 V. Chobot, F. Hadacek and L. Kubicova, Effects of selected dietary secondary metabolites on reactive oxygen species production caused by iron(II) autoxidation, *Molecules*, 2014, **19**, 20023–20033.
- 642 J. R. O'Dell, G. Paulsen, C. E. Haire, K. Blakely, W. Palmer, S. Wees, P. J. Eckhoff, L. W. Klassen, M. Churchill, D. Doud, A. Weaver and G. F. Moore, Treatment of early seropositive rheumatoid arthritis with minocycline: four-year followup of a double-blind, placebo-controlled trial, *Arthritis Rheum.*, 1999, **42**, 1691–1695.



- 643 R. M. Bonelli, A. K. Hodl, P. Hofmann and H. P. Kapfhammer, Neuroprotection in Huntington's disease: a 2-year study on minocycline, *Int. Clin. Psychopharmacol.*, 2004, **19**, 337–342.
- 644 T. Miyaoka, R. Yasukawa, H. Yasuda, M. Hayashida, T. Inagaki and J. Horiguchi, Minocycline as adjunctive therapy for schizophrenia: an open-label study, *Clin. Neuropharmacol.*, 2008, **31**, 287–292.
- 645 F. Zhao, Y. Hua, Y. He, R. F. Keep and G. Xi, Minocycline-induced attenuation of iron overload and brain injury after experimental intracerebral hemorrhage, *Stroke*, 2011, **42**, 3587–3593.
- 646 T. Miyaoka, R. Wake, M. Furuya, K. Liaury, M. Ieda, K. Kawakami, K. Tsuchie, M. Taki, K. Ishihara, T. Araki and J. Horiguchi, Minocycline as adjunctive therapy for patients with unipolar psychotic depression: an open-label study, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2012, **37**, 222–226.
- 647 C. E. Schmitt, S. G. Fabi, T. Kukreja and J. S. Feinberg, Hypopigmented cutaneous sarcoidosis responsive to minocycline, *J. Drugs Dermatol.*, 2012, **11**, 385–389.
- 648 J. K. Soczynska, R. B. Mansur, E. Brietzke, W. Swardfager, S. H. Kennedy, H. O. Woldeyohannes, A. M. Powell, M. S. Manierka and R. S. McIntyre, Novel therapeutic targets in depression: minocycline as a candidate treatment, *Behav. Brain Res.*, 2012, **235**, 302–317.
- 649 S. F. Abcouwer, C. M. Lin, S. Shanmugam, A. Muthusamy, A. J. Barber and D. A. Antonetti, Minocycline prevents retinal inflammation and vascular permeability following ischemia-reperfusion injury, *J. Neuroinflammation*, 2013, **10**, 149.
- 650 Y. Li, T. Li, H. Qi and F. Yuan, Minocycline protects against hepatic ischemia/reperfusion injury in a rat model, *Biomed. Rep.*, 2015, **3**, 19–24.
- 651 N. Garrido-Mesa, A. Zarzuelo and J. Gálvez, Minocycline: far beyond an antibiotic, *Br. J. Pharmacol.*, 2013, **169**, 337–352.
- 652 N. Garrido-Mesa, A. Zarzuelo and J. Gálvez, What is behind the non-antibiotic properties of minocycline?, *Pharmacol. Res.*, 2013, **67**, 18–30.
- 653 T. G. Marshall and F. E. Marshall, Sarcoidosis succumbs to antibiotics – implications for autoimmune disease, *Autoimmun. Rev.*, 2004, **3**, 295–300.
- 654 L. Xie, L. Xie and P. E. Bourne, Structure-based systems biology for analyzing off-target binding, *Curr. Opin. Struct. Biol.*, 2011, **21**, 189–199.
- 655 J. Ochoa-Repáraz, D. W. Mielcarz, L. E. Ditrío, A. R. Burroughs, D. M. Foureau, S. Haque-Begum and L. H. Kasper, Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis, *J. Immunol.*, 2009, **183**, 6041–6050.
- 656 H. Yokote, S. Miyake, J. L. Croxford, S. Oki, H. Mizusawa and T. Yamamura, NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora, *Am. J. Pathol.*, 2008, **173**, 1714–1723.
- 657 J. Ochoa-Repáraz, D. W. Mielcarz, S. Begum-Haque and L. H. Kasper, Gut, bugs, and brain: role of commensal bacteria in the control of central nervous system disease, *Ann. Neurol.*, 2011, **69**, 240–247.
- 658 K. Berer, M. Mues, M. Koutrolos, Z. A. Rasbi, M. Boziki, C. Johner, H. Wekerle and G. Krishnamoorthy, Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination, *Nature*, 2011, **479**, 538–541.
- 659 K. Berer and G. Krishnamoorthy, Commensal gut flora and brain autoimmunity: a love or hate affair?, *Acta Neuropathol.*, 2012, **123**, 639–651.
- 660 Y. Wang and L. H. Kasper, The role of microbiome in central nervous system disorders, *Brain, Behav., Immun.*, 2014, **38**, 1–12.
- 661 J. Ochoa-Repáraz and L. H. Kasper, Gut microbiome and the risk factors in central nervous system autoimmunity, *FEBS Lett.*, 2014, **588**, 4214–4222.
- 662 D. Astrauskiene and E. Bernotiene, New insights into bacterial persistence in reactive arthritis, *Clin. Exp. Rheumatol.*, 2007, **25**, 470–479.
- 663 M. Ogrendik and N. Karagoz, Treatment of rheumatoid arthritis with roxithromycin: a randomized trial, *Postgrad. Med.*, 2011, **123**, 220–227.
- 664 B. Kwiatkowska and M. Maślińska, Macrolide therapy in chronic inflammatory diseases, *Mediators Inflammation*, 2012, **2012**, 636157.
- 665 M. Ogrendik, Rheumatoid arthritis is an autoimmune disease caused by periodontal pathogens, *Int. J. Gen. Med.*, 2013, **6**, 383–386.
- 666 V. N. Saxena and J. Dogra, Long-term use of penicillin for the treatment of chronic plaque psoriasis, *Eur. J. Dermatol.*, 2005, **15**, 359–362.
- 667 V. N. Saxena and J. Dogra, Long-term oral azithromycin in chronic plaque psoriasis: a controlled trial, *Eur. J. Dermatol.*, 2010, **20**, 329–333.
- 668 A. A. Alzolibani and K. Zedan, Macrolides in Chronic Inflammatory Skin Disorders, *Mediators Inflammation*, 2012, 159354.
- 669 A. Vila-Corcoles, O. Ochoa-Gondar, T. Rodriguez-Blanco, A. Gutierrez-Perez, A. Vila-Rovira, F. Gomez, X. Raga, C. de Diego, E. Satue, E. Salsench and EPIVAC Study Group, Clinical effectiveness of pneumococcal vaccination against acute myocardial infarction and stroke in people over 60 years: the CAPAMIS study, one-year follow-up, *BMC Public Health*, 2012, **12**, 222.
- 670 A. Vila-Corcoles, O. Ochoa-Gondar, T. Rodriguez-Blanco, C. de Diego-Cabanes, E. Satue-Gracia, A. Vila-Rovira, C. Torrente Fraga and E. R. Group, Evaluating clinical effectiveness of pneumococcal vaccination in preventing stroke: the CAPAMIS Study, 3-year follow-up, *J. Stroke Cerebrovasc. Dis.*, 2014, **23**, 1577–1584.
- 671 J. van der Greef, T. Hankemeier and R. N. McBurney, Metabolomics-based systems biology and personalized medicine: moving towards  $n = 1$  clinical trials?, *Pharmacogenomics*, 2006, **7**, 1087–1094.
- 672 N. B. Gabler, N. Duan, S. Vohra and R. L. Kravitz,  $N$ -of-1 trials in the medical literature: a systematic review, *Med. Care*, 2011, **49**, 761–768.
- 673 X. Chen and P. Chen, A comparison of four methods for the analysis of  $N$ -of-1 trials, *PLoS One*, 2014, **9**, e87752.



- 674 H. H. Kong, J. Oh, C. Deming, S. Conlan, E. A. Grice, M. A. Beatson, E. Nomicos, E. C. Polley, H. D. Komarow, N. C. S. Program, P. R. Murray, M. L. Turner and J. A. Segre, Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis, *Genome Res.*, 2012, **22**, 850–859.
- 675 H. R. Schumacher Jr., R. R. Evans, K. G. Saag, J. Clower, W. Jennings, S. P. Weinstein, G. D. Yancopoulos, J. Wang and R. Terkeltaub, Rilonacept (interleukin-1 trap) for prevention of gout flares during initiation of uric acid-lowering therapy: results from a phase III randomized, double-blind, placebo-controlled, confirmatory efficacy study, *Arthritis Care Res.*, 2012, **64**, 1462–1470.
- 676 K. L. Molnar-Kimber and C. T. Kimber, Each type of cause that initiates rheumatoid arthritis or RA flares differentially affects the response to therapy, *Med. Hypotheses*, 2012, **78**, 123–129.
- 677 V. P. Bykerk, E. Lie, S. J. Bartlett, R. Alten, A. Boonen, R. Christensen, D. E. Furst, S. Hewlett, A. L. Leong, A. Lyddiatt, L. March, J. E. May, P. Montie, A. M. Orbai, C. Pohl, M. Scholte Voshaar, T. Woodworth, C. O. Bingham 3rd and E. H. Choy, Establishing a core domain set to measure rheumatoid arthritis flares: report of the OMERACT 11 RA flare Workshop, *J. Rheumatol.*, 2014, **41**, 799–809.
- 678 R. Zhang, N. F. Lahens, H. I. Ballance, M. E. Hughes and J. B. Hogenesch, A circadian gene expression atlas in mammals: Implications for biology and medicine, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 16219–16224.
- 679 K. de Punder and L. Pruimboom, Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability, *Front. Immunol.*, 2015, **6**, 223.

