Review Article
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The Immunopathology of Sepsis: Pathogen Recognition, Systemic Inflammation, the Compensatory Anti-Inflammatory Response, and Regulatory T Cells

D.H. Lewis, D.L. Chan, D. Pinheiro, E. Armitage-Chan, and O.A. Garden

Sepsis, the systemic inflammatory response to infection, represents the major cause of death in critically ill veterinary patients. Whereas important advances in our understanding of the pathophysiology of this syndrome have been made, much remains to be elucidated. There is general agreement on the key interaction between pathogen-associated molecular patterns and cells of the innate immune system, and the amplification of the host response generated by pro-inflammatory cytokines. More recently, the concept of immunoparalysis in sepsis has also been advanced, together with an increasing recognition of the interplay between regulatory T cells and the innate immune response. However, the heterogeneous nature of this syndrome and the difficulty of modeling it *in vitro* or *in vivo* has both frustrated the advancement of new therapies and emphasized the continuing importance of patient-based clinical research in this area of human and veterinary medicine.

Key words: Clinical pathology; Critical care; Hematology; Immunology; Lymphocyte function; Multiple organ failure; Shock.

Sepsis, defined as the systemic inflammatory response to infection (Box 1), remains the major cause of death in critically ill human patients.[1–6] Recent human studies estimate the annual incidence of sepsis to be 240–300 cases per 100,000 population, with associated costs of nearly $17 billion in the United States[7,8]; the rate of occurrence is also increasing at around 9% each year.[8] Although large-scale veterinary epidemiological studies are uncommon, a substantial proportion of the critically ill veterinary population is estimated to be septic.[9,10] The case fatality rate associated with sepsis in a variety of veterinary species is reported to approach 50%, emphasizing the need for a greater understanding of the pathophysiology of sepsis to improve therapeutic practices.[11–13]

The pathophysiology of sepsis remains incompletely understood. A multitude of cell types, inflammatory mediators, and coagulation factors are involved and recent research has focused on the contributions of the innate immune system and T cells in this complex syndrome.[2,14–20] This review will present an account of the current understanding of the function of the immune system in sepsis, with emphasis on the interaction of pathogens with innate components of the immune system and the key role of the endothelium in triggering and propagating a pro-inflammatory state.

From the Department of Veterinary Clinical Sciences, The Royal Veterinary College, Hatfield Campus, Hertfordshire, UK (Lewis, Chan, Garden); Regulatory T Cell Laboratory, The Royal Veterinary College, Camden Campus, London, NW1 OTU, UK (Pinheiro, Garden); Davies Veterinary Specialists, Manor Farm Business Park, Hertfordshire, SG5 3HR, UK (Armitage-Chan); and DHL's current address: Langford Veterinary Services, Small Animal Hospital, Langford House, Langford, Bristol, BS40 5DU, UK.

Corresponding author: O.A. Garden, Department of Veterinary Clinical Sciences, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK; e-mail: ogarden@rvc.ac.uk.

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Abbreviations:

AGE	advanced glycation end product
AP-1	activator protein 1
aPC	activated protein C
BK	bradykinin
CARD	common caspase activation and recruitment domain
CLARP	caspase-like apoptosis regulatory protein
DAMP	danger-associated molecular pattern
gp	glycoprotein
HMBG	high mobility group box protein
HSP	heat shock protein
Ig	immunoglobulin
IKK	inhibitor of κ B kinase
IPAF	interleukin-1 converting enzyme (ICE) protease-activating factor
IPS-1	interferon-β promoter stimulator-1 (also known as CARD adaptor inducing interferon-β [Cardif])
IRAK	IL-1R associated kinase
IRF	interferon regulatory factor
KK	kallikrein
LDL	low density lipoprotein
LGP2	laboratory of genetics and physiology 2
MAPK	mitogen-activated protein kinase
MDA5	melanoma differentiation-associated gene 5
MODS	multiple organ dysfunction syndrome
MyD88	myeloid differentiation primary response gene 88
NAIP	neuronal inhibitor of apoptosis
NEMO	NF-κB essential modulator
NF-κB	nuclear factor kappa B
NLR	nucleotide-binding domain, leucine-rich repeat containing protein
NLRB	NLR family, baculovirus inhibitor of apoptosis protein repeat domain containing
NLRC	NLR family, CARD containing
NLRP	NLR family, pyrin domain containing
NOD	nucleotide-binding oligomerization domain
PAMP	pathogen-associated molecular pattern
PAR	protease-activated receptor
PMN	polymorphonuclear cell
PRR	pattern-recognition receptor
RAGE	receptor for advanced glycation end products
RICK	RIP-like interacting CLARP kinase
RIG-1	retinoic acid-inducible gene-1

Abbreviations:

AGE	advanced glycation end product
AP-1	activator protein 1
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CARD	common caspase activation and recruitment domain
CLARP	caspase-like apoptosis regulatory protein
DAMP	danger-associated molecular pattern
gp	glycoprotein
HMBG	high mobility group box protein
HSP	heat shock protein
Ig	immunoglobulin
IKK	inhibitor of κ B kinase

Box 1
Definitions of SIRS, sepsis, and MODS[1,4,6]
SIRS (systemic inflammatory response syndrome): clinical manifestation of the systemic response to injury.

Sepsis: SIRS in association with bacterial, viral, protozoal or fungal infection.

Severe sepsis: sepsis together with evidence of organ dysfunction, hypoperfusion, or hypotension.

Septic shock: sepsis with hypotension despite adequate fluid resuscitation.

MODS (multiple organ dysfunction syndrome): altered organ function in an acutely ill patient such that homeostasis cannot be maintained.

In addition, the complex interplay between pro- and anti-inflammatory cytokines and the spectrum of the host defense response will be discussed. Finally, the importance of regulatory T cells (Tregs) in maintaining the balance of host inflammatory mechanisms will be described.

Immunopathology of Sepsis

Living organisms face a constant barrage of potentially pathogenic microorganisms. Survival depends upon physical barriers to resist entry of pathogens, as well as the presence of a constitutive, or innate, immune system that can rapidly induce a defensive inflammatory response. Such a system can be found in virtually all species, suggesting that it is evolutionarily ancient and highly successful.[21,22] The innate immune system further interacts with the adaptive immune system, based on a system of T and B lymphocytes that respond to specific epitopes.

Surface Barrier Mechanisms and Antimicrobial Peptides

Whereas the keratinized epithelium of the dermis and the mucous lining of the body cavities discourage pathogenic colonization, various other innate defenses are employed to minimize penetration of the body wall by microorganisms, including antimicrobial peptides (AMPs; otherwise known as host-defense peptides) on mucosal surfaces.[23,24] A comprehensive review of AMPs in veterinary species has recently been published.[25] In summary, AMPs comprise 3 main groups: digestive enzymes and peptides that disrupt the microbial cell membrane, peptides that bind essential elements, and peptides that act as decoys for microbial attachment. The 2 major classes of bactericidal AMPs in the mammalian immune system are the defensins and cathelicidins. Compared to eukaryotic cells, bacterial cell walls lack cholesterol—which acts to stabilize cell membranes—and negatively charged phospholipids. Instead, bacterial cell walls are rich in anions (basic amino acids) and thus attract the cationic defensins, which carpet the microbial membrane and institute channel formation.[26] These channels then lead to transmembrane pore formation, membrane destabilization, and microbial cell death, although recent studies suggest that their function could go beyond that of lipid bilayer perturbation.[27] Whereas a large number of AMPs form part of the non-oxidative killing mechanism within phagolysosomes in cells such as neutrophils and macrophages, a growing number are thought to be actively secreted onto epithelial surfaces of the gastrointestinal, respiratory and urinary tracts[28–31]—including the ovine gastrointestinal tract[32] and the canine testis.[33] Recent work has revealed that important amounts of these AMPs are secreted not only by immune cells such as neutrophils and alveolar macrophages but also by atypical defense cells such as type II pneumocytes.[34] This theme is universal throughout the processes of the innate immune system and challenges the traditional view of a "standing army" of immune defense cells, replacing it with the concept of a body-wide, integrated community of cells contributing to pathogen vigilance.[35]

Recognition of Pathogens: PAMPs, MAMPs and DAMPs

Two factors are vital to the rapid ability of the innate immune system to respond to pathogen incursion: the presence of receptors against pathogen markers and the ubiquitous nature of these receptors in the body.[35] Individual receptors are genetically encoded and display strong homology within and between species.[22] These receptors are not only expressed on many effector cells of the immune system—including macrophages, neutrophils, dendritic cells, and lymphocytes—but are also found on epithelial cells, endothelial cells, and myocytes[36,37]; expression has also been detected in the bovine endometrium.[38] The major targets of these pattern recognition receptors (PRRs) are known as pathogen-associated molecular patterns (PAMPs), although the presence of these molecules in nonpathogenic and commensal bacteria has led to the suggestion that the term "microbial-associated molecular

patterns" (MAMPs) is more accurate. These molecules share certain core characteristics:

- PAMPs are produced only by microbial pathogens, not by the host (eg, peptidoglycans are produced by bacteria but not by eukaryotic cells): this confers automatic self/nonself discriminatory ability.
- PAMPs are generally invariant molecules shared by entire classes of microorganisms (eg, lipoarabinomannan is found on the cell wall of all *Mycobacteria*); this allows the evolutionary retention of a relatively small number of PRRs recognizing vast numbers of potential pathogens.
- PAMPs are usually structures vital to the survival or pathogenicity of the microorganism (eg, lipopolysaccharide [LPS] in the outer membrane of Gram-negative bacteria): this allows targeting of highly conserved molecules and obviates the need for variability in host PRRs.

Although bacterial cell surface components such as LPS in the outer cell membrane of Gram-negative bacteria and lipotechoic acid (LTA) in the cell membrane of Gram-positive bacteria represent classic examples of PAMPs, recognition of "altered self" secondary to host cell colonization by viral pathogens is also likely. The innate immune system has thus developed the ability to detect markers of endogenous cell damage called "alarmins" or "danger-associated molecular patterns" (DAMPs)[39–41] Table 1 shows key PAMPs and their corresponding receptors,[42,43] whereas Table 2 shows some of the confirmed interactions of DAMPs.[39,40,43–46] Nonrodent sepsis models, genetic approaches, and immunological studies have demonstrated the presence of a large number of PRRs in clinical veterinary species (Table 3).[38,47–55]

Initiation of an inflammatory reaction to necrotic, rather than apoptotic, cell death would appear to be useful in host defense; however, the interaction of DAMPs and PAMPs with their receptors leads to increased case fatality rates in sepsis.[15,19,56] Owing to PRR cross-reactivity for both PAMPs and DAMPs, multiple positive feedback systems become established, leading to rapid progression of a global inflammatory response with consequent clinical signs (Fig 1).[16,19,57] Functional interactions between PRRs, including synergy and cross-tolerance, also occur.[58,59] If an inflammatory state persists, the very defensive mechanisms of the innate immune system designed to protect the host can lead to further tissue damage, as well as diminished antimicrobial activity that allows opportunistic secondary infections.[15,20] Whereas the existence of such feedback systems in veterinary species can at present only be inferred, experimental data suggest PRR reactivity to both pathogen- and host-derived ligands in cattle, pigs, horses, and dogs.[60–66] Furthermore, the blunted PAMP-induced TNF, IL-6 and IL-10 response of whole blood in dogs with lymphoma is thought to underlie their higher risk of sepsis.[67]

Table 1. Key pathogen-associated molecular pattern ligands of the pattern recognition receptors implicated in sepsis.

PRR	Location	Cell Type	PAMP Recognized
TLR 1/CD281	pm	pbmc, np, u	triacyl lipopeptide
TLR 2/CD282	pm, el	pbmc, dc, mc, nkc	peptidoglycan, lipoprotein, lipopolysaccharide, glycosylphosphatidylinositols, mannans
TLR 3/CD283	pm, el	dc, epi, fb, blc, nkc	ssRNA, dsRNA, dsDNA
TLR 4/CD284	pm	pbmc, mc, np, epi	lipopolysaccharide, glycosylphosphatidylinositols, viral envelope proteins, mannans
TLR 5	pm	pbmc, dc, epi, nkc	flagellin
TLR 6/CD286	pm	mc, blc	diacyl lipopeptide
TLR 7	el	pbmc, dc, blc	ssRNA
TLR 8/CD288	el	nkc	ssRNA
TLR 9/CD289	el	dc, blc, nkc, epi	ssDNA, dsDNA
TLR 10/CD290	pm	pbmc, dc, blc	Unknown
TLR 11	pm	epi, dc, pbmc	profilin
NOD1/NLRC1	cyt, pm	epi, dc, pbmc	peptidoglycan
NOD2/NLRC2/CARD15	cyt, pm	epi, dc, pbmc, Paneth cells	muramyl dipeptide
NLRC4/IPAF	cyt	unknown	flagellin
NLRP1	cyt	unknown	muramyl dipeptide, *Bacillus anthracis* lethal toxin
NLRP3	cyt	unknown	bacterial & viral RNA, lipopolysaccharide, lipotechoic acid, muramyl dipeptide
NLRB1/NAIP5	cyt	unknown	flagellin

PRR, pattern-recognition receptor; PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor; NOD, nucleotide-binding oligomerization domain; NLR, nucleotide-binding domain, leucine-rich repeat containing protein; NLRC, NLR family, CARD containing; IPAF, interleukin-1 converting enzyme (ICE) protease-activating factor; NLRP, NLR family, pyrin domain containing; NLRB, NLR family, baculovirus inhibitor of apoptosis protein repeat domain containing; NAIP, neuronal inhibitor of apoptosis; pm, plasma membrane; el, endolysosomes; cyt, cytoplasma; np, neutrophils; u, ubiquitous; dc, dendritic cells; mc, mast cells; nkc, natural killer cells; epi, epithelial cells; fb, fibroblasts; blc, B lymphocytes; ss, single-stranded; ds, double-stranded.

Table 2. Key danger-associated molecular pattern ligands of the pattern recognition receptors.

PRR	Location	Cell Type	DAMP Recognized
TLR 2/CD282	pm, el	pbmc, dc, mc, nkc	HMGB 1, necrotic cells, HSP-60, HSP-70, gp-96, biglycan, defensins
TLR 3/CD283	pm, el	dc, epi, fb, blc, nkc	endogenous mRNA
TLR 4/CD284	pm	pbmc, mc, np, epi	HSP-22, HSP-70, HSP-90, fibronectin, fibrinogen, heparan fragments, hyaluronate fragments, β-defensin 2, oxidized LDL, surfactant protein A, neutrophil elastase, HMGB 1, biglycan
TLR 9/CD289	el	dc, blc, nkc, epi	chromatin-IgG complex
NLRP3	cyt	unknown	uric acid crystals
RAGE	pm	u?	AGEs, HMGB 1, amyloid peptide, S100s

New abbreviations (for previous abbreviations see Table 1): DAMP, danger-associated molecular pattern; RAGE, receptor for advanced glycation end products; HMGB, high mobility group box protein; HSP, heat shock protein; gp, glycoprotein; LDL, low density lipoprotein; Ig, immunoglobulin; AGEs, advanced glycation end products; S100s, S100 proteins (calgranulins).

Table 3. Pattern-recognition receptors of those families implicated in sepsis identified in veterinary species to date.

Species	TLRs	NLRs
Dog	1–7, 9	NOD1, NOD2, NLRC4 NLRP 1–3, 5, 6, 8–10, 12–14
Cat	2–5, 7–9	
Horse	2–4, 6, 9	NLRC4
Cow	1–10	NOD1, NOD2, NLRC4 NLRP 1, 3, 5, 6, 8–10, 12–14
Sheep	1–10	NOD2
Goat	1–10	
Pig	1–10	NOD1, NOD2

For abbreviations, see footnote to Table 1.

Table 4. The spectrum of type I and type II acute phase proteins.

Acute Phase Protein	Role	Type
Serum amyloid A	Leukocyte recruitment and activation	I – induced by IL-1 and TNF
C-reactive protein	Enhance microbial phagocytosis and complement binding	
C3, C4, C4BP, C1inh	Complement components	
Haptoglobin (rat)	Binds free hemoglobin	
α1-acid glycoprotein	Transport functions	
Fibrinogen	Hemostasis	II – induced by IL-6 and IL-6-like cytokines
Haptoglobin (man)	Binds free hemoglobin	
(Apo)Ferritin	Binds free iron	
α1-antitrypsin	Protease inhibitor	
α2-macroglobulin	Protease inhibitor	

Pattern Recognition Receptors: an Overview

Evolutionary pressure has resulted in the encoding of a number of different host proteins within three distinct families—the Toll-like receptors (TLRs), the nucleotide-binding oligomerization domain, leucine-rich repeat containing proteins (NLRs; previously designated as the nucleotide-binding oligomerization domain [NOD]-like receptors) and the retinoic acid-inducible gene-1 (RIG-1)-like receptors (RLRs).[68–70] Cooperation between the RLRs—intracellular viral nucleic acid sensors—and the endosomal TLRs appears likely,[71–73] although little is currently known about the involvement of RLRs in the pathogenesis of sepsis.[14] This family of PRRs is therefore not considered further in this review.

Whereas PRRs form a heterogenous group of proteins, certain characteristics—such as leucine-rich repeat domains, scavenger receptor cysteine-rich domains, and C-type lectin domains—can be commonly recognized.[68,69]

The Toll-Like Receptors

The Toll gene was first identified in *Drosophila melanogaster* as encoding a transmembrane glycoprotein with a role in determination of dorsoventral polarity in the embryo.[74] Additional investigation revealed a large extracellular component with leucine-rich repeats, with a cytoplasmic portion described as the TIR (Toll/interleukin-1 receptor [L-1R]) domain owing to its similarity to the intracellular region of the mammalian IL-1R.[75] Not only was the Toll protein discovered to play a role in the innate immune response to fungal infection in *Drosophila*, but a subsequent series of studies served to highlight the link between TLRs and the mammalian immune response.[76–79] To date, 10 human and 12 murine functional TLRs have been identified by a combination of immunological techniques and examination of genetic databases.[42,71]

The cytoplasmic TIR domain of the TLRs interacts with a variety of TIR-domain-containing adaptors (Fig 2).[80–83] PAMP/DAMP ligation of the respective PRR activates a chain of kinases in the IL-1R associated kinase (IRAK) family, ultimately resulting in activation of the inhibitor of κ B kinase (IKK) enzyme complex and the mitogen-activated protein kinase (MAPK) pathway (Fig 2).[84–86] Whereas the connection of TLR activation to the NF-κB and MAPK signaling pathways is well recognized, a number of other pathways are also triggered by TLR stimulation, including Protein Kinase R/eukaryotic translation initiation

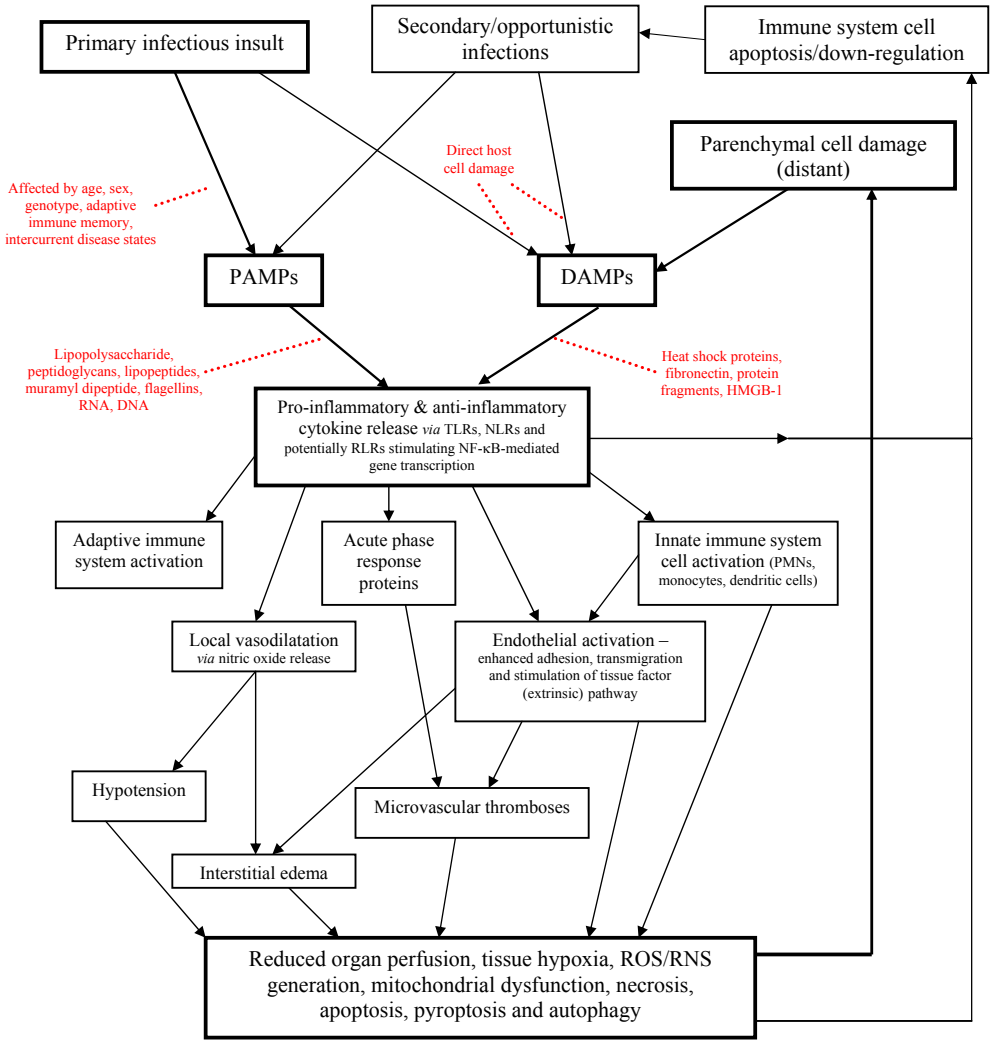

Fig 1. The pathophysiology of sepsis—an overview. A primary infectious insult—of bacterial, viral, protozoal or fungal origin—damages host tissues. The ligation of pattern-recognition receptors (TLRs and NLRs) by PAMPs and DAMPs promotes the release of both pro- and anti-inflammatory cytokines, as well as acute phase proteins, with a number of pathophysiological sequelae that ultimately lead to organ hypoperfusion, tissue hypoxia, the generation of ROS and RNS, mitochondrial dysfunction and cell death by necrosis, apoptosis, pyroptosis, and autophagy. Further DAMPs are generated, helping to perpetuate the inflammatory response, and the death of immune cells leaves the organism vulnerable to secondary, or opportunistic, infections. Abbreviations: PAMPs = pathogen-associated molecular patterns; DAMPs = danger-associated molecular patterns; TLRs = Toll-like receptors; NLRs = nucleotide-binding domain, leucine-rich repeat containing protein; RLR = retinoic acid-inducible gene-1 (RIG-1)-like receptor; NFκB = nuclear factor kappa B; PMN = polymorphonuclear cell; ROS = reactive oxygen species; RNS = reactive nitrogen species.

factor 2-α kinase 2 (PKR/elF2α), Notch, phosphoinositide 3-kinases (PI-3K), and small GTPases.[87–92]

TLRs are expressed by a number of different cells, including dendritic cells, macrophages, B cells, natural killer (NK) cells, endothelial cells, epithelial cells, and fibroblasts (Tables 1 and 2); their site of expression varies, with TLRs 1, 2, 4, 5, 6, and 11 present on the external plasma membrane and TLRs 3, 7, 8, and 9 in endosomes.[71,93]

TLR 2 appears to be of key importance, owing to its ability to recognize PAMPs as diverse as lipotechoic acid (from Gram-positive bacteria), peptidoglycan (from Gram-positive and Gram-negative bacteria), hemagglutinin (from measles virus), polysaccharides (from yeasts), lipoproteins (from *E. coli*, *Borrelia burgdorferi*, *Mycoplasma* spp. and *Mycobacterium tuberculosis*), as well as complete pathogens such as *Clostridium* spp., *Chlamydophila* spp., and herpes simplex virus, and a variety of endogenous ligands.[94–97] Much of its wide-ranging influence stems from the formation of heterodimers with TLRs 1 and 6.[98–100] TLR 4 also has a significant role in triggering the innate immune response as it recognizes molecules such as LPS (from Gram-negative bacteria), various viral protein envelopes, and a large number of endogenous molecules (Table 2).[57,101–103] The vital recognition of LPS by TLR

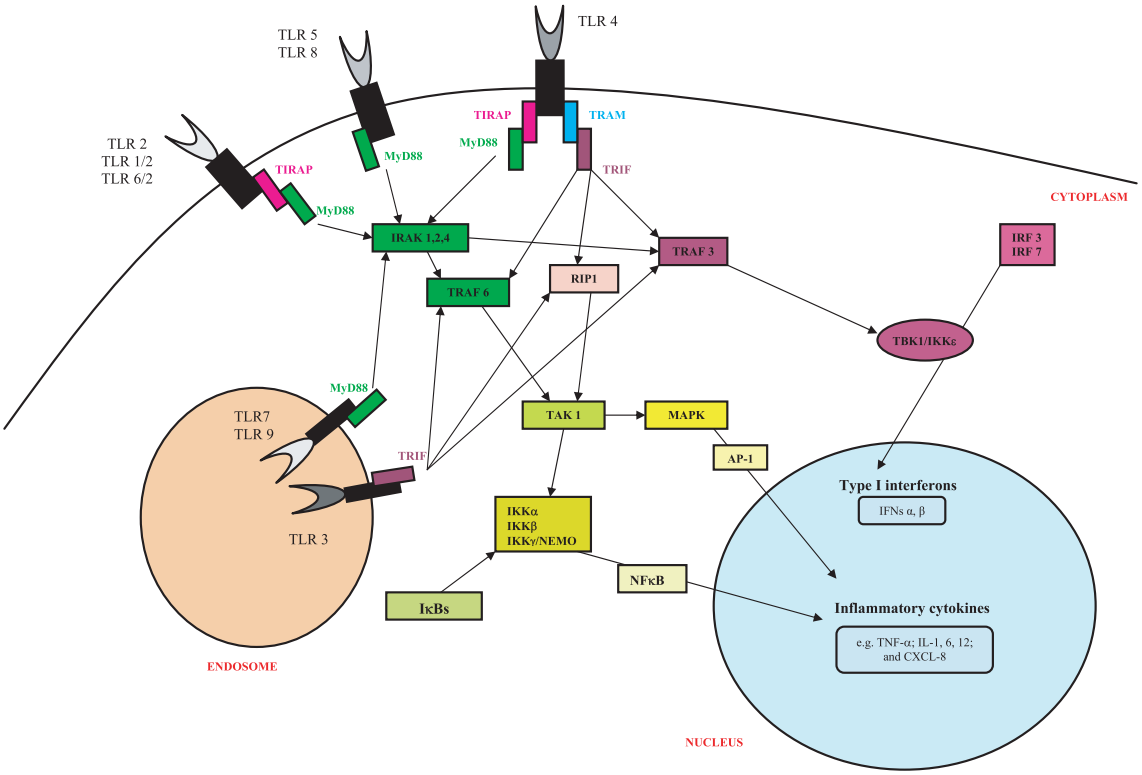

Fig 2. Toll-like receptor (TLR) signaling pathways. The cytoplasmic Toll/interleukin-1 receptor (TIR) domain of the TLRs interacts with TIR-domain-containing adaptors, such as myeloid differentiation primary response gene 88 (MyD88), TIR-containing adaptor protein (TIRAP), TIR-domain-containing adaptor molecule 1 (TICAM1, also known as TRIF) and TIR-domain-containing adaptor molecule 2 (TICAM2, also known as TRAM). PAMP binding to the respective receptor results in the activation of either the MyD88 or TICAM1/TRIF signaling pathways. These pathways involve a series of kinases in the IL-1R associated kinase (IRAK) family, whose action eventually results in activation of the inhibitor of κ B kinase (IKK) enzyme complex and the mitogen-activated protein kinase (MAPK) pathway. The IKK complex phosphorylates the inhibitory IκBα protein, thus freeing the nuclear transcription factor nuclear factor kappa B (NF-κB); triggering of the MAPK signaling pathway results in the activation of activator protein 1 (AP-1). Both NF-κB and AP-1 then initiate the transcription of cytokine genes. Abbreviations: TRAF = TNF receptor-associated factor; RIP = receptor interacting protein; TAK = TGF-β-activated kinase; NEMO = NF-κB essential modulator; TBK = TRAF family member-associated NF-κB activator (TANK)-binding kinase; IRF = interferon regulatory factor.

4 appears to be dependent on formation of a complex with other PRRs, myeloid differentiation protein-2 (MD2), membrane-bound CD14 (mCD14), and lipopolysaccharide-binding protein (LBP).[71,104] Both TLR 2 and 4 have been extensively researched in human septic patients, in whom early upregulation of these TLRs can exacerbate the severity of illness and mortality.[19,57]

TLR 5 is expressed by epithelial cells of the respiratory and intestinal tract and is a PRR for flagellin, an important component of motile bacteria such as *Salmonella* spp.[105,106] TLR 5 is incriminated in sepsis,[107] although exactly how the PAMP and PRR interact *in vivo* remains unclear.[108] The intracellular TLRs 3, 7, 8, and 9 all share high sequence homology and recognize nucleotides.[71,109] TLR 3 is the only TLR yet discovered that does not initiate the MyD88 signaling pathway, interacting solely with TIR-domain-containing adaptor molecule 1 (TICAM1, also known as TRIF) (Fig 2).[109,110] TLR 11, to date only identified in rats and mice, appears to recognize *Toxoplasma gondii* and certain pathogens of the urinary tract, although a role in sepsis has not so far been described.[111,112]

The Nucleotide-Binding Domain, Leucine-Rich Repeat Containing Proteins, and Inflammasomes

The realization that TLRs could not account for the full range of PAMP recognition motivated the discovery of additional intracellular PRRs, including the NLRs. Of the NLRs, the 2 cytosolic receptors NOD1 and NOD2 were the first to be discovered; subsequent examination of genomic databases has suggested that there are at least 23 NLRs in humans and 34 in mice.[113–117] Common to all NLRs is their structure, comprising a leucine-rich repeat domain (thought to be the PAMP receptor region), a central NOD domain, and an N-terminal effector domain responsible for downstream signaling.[118–120] The NLRs are found in the cytosolic compartment of eukaryotic cells, although some recent evidence suggests that they can also be associated with the plasma membrane.[121,122] The mechanisms underlying NLR contact with their respective PAMPs have yet to be confirmed *in vivo*,[123–125] but recognition of the PAMP triggers the activation of a series of kinases leading to the phosphorylation of

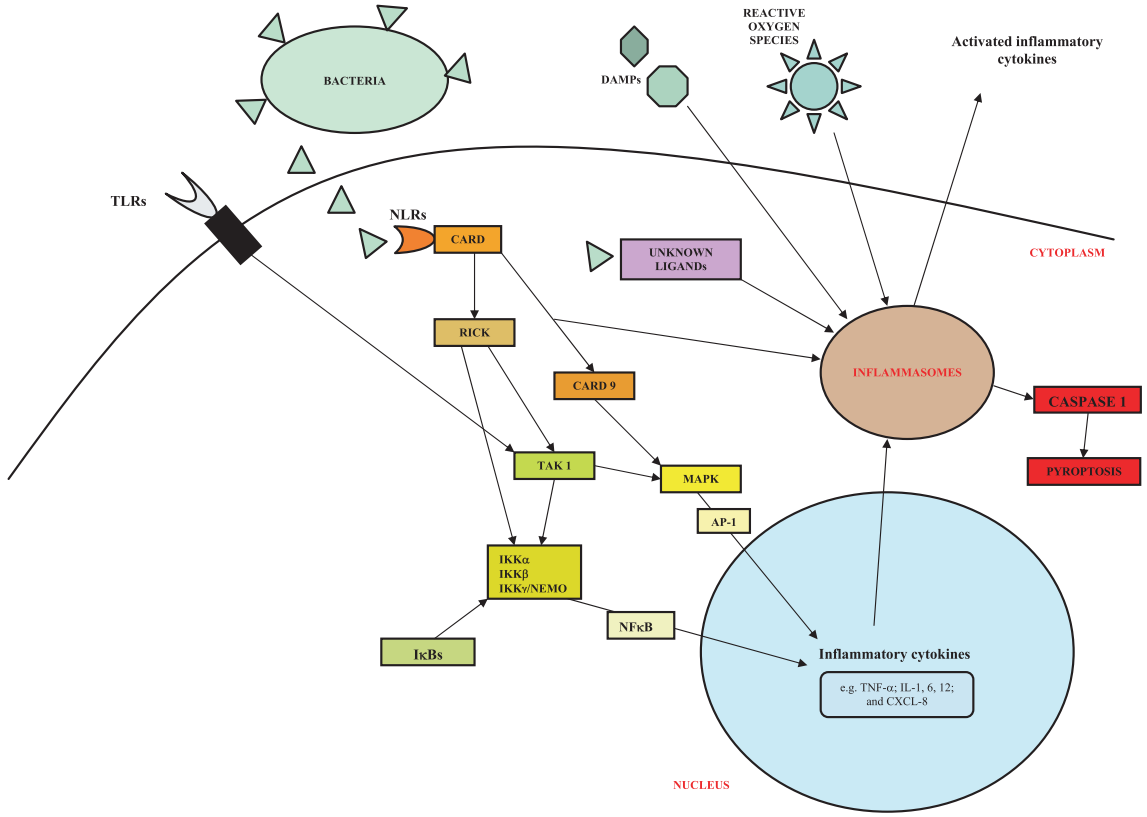

Fig 3. Nucleotide-binding domain, leucine-rich repeat containing protein (NLR) signaling pathways. Upon PAMP ligation, NLRs recruit the serine-threonine kinase RIP-like interacting caspase-like apoptosis regulatory protein (CLARP) kinase (RICK), also known as receptor-interacting serine/threonine-protein kinase 2 (RIPK2 or RIP2), which binds to NF-κB essential modulator (NEMO), a subunit of IKK, resulting in the phosphorylation of IκB and the release of NF-κB; RICK also mediates the recruitment of transforming growth factor β-activated kinase 1 (TAK1) and together these molecules stimulate activation of the mitogen-activated protein kinase (MAPK) signaling pathway. In common with the TLR family, NLR activation leads to the transcription of inflammatory cytokine genes via the mobilization of NF-κB and AP-1. Another important consequence of NLR ligation is activation of the inflammasome, a macromolecular complex comprising pro-caspase-1 together with various adapter proteins. Caspase-1 is important in the synthesis of active IL-1β and IL-18, and induces a type of programmed cell death called pyroptosis.

IκB, mobilization of NF-κB, and activation of MAPK signaling (Fig 3).[126–129] NOD1 (NLR family, caspase activation, and recruitment domain [CARD] containing 1; NLRC1) and NOD2 (NLRC2, also known as CARD15) are known to recognize muropeptides derived from peptidoglycan, a major structural component of both Gram-positive and Gram-negative bacterial cell walls (Table 1).[115,118] Whereas the role of NOD2 is well established in the pathogenesis of Crohn's disease,[130] little is known about the role of the NOD proteins in sepsis. However, synthetic NOD1 agonists administered to mice stimulate chemokine production, neutrophil recruitment,[131] and—in one model—shock and multiple organ dysfunction,[132] raising the possibility of NOD1 involvement in the pathogenesis of sepsis.

A number of studies have been performed on the role of inflammatory cysteinyl aspartate-specific proteinases, or caspases, in murine and human sepsis. Caspase-1 has been the focus of particular attention and is activated by a macromolecular complex of around 700 kDa—one of a number of inflammasomes[133]—comprising pro-caspase-1 together with various adapter proteins, including apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC).[133–135] Activation of the currently identified inflammasomes depends upon the interaction of NLR family members (NLRC4; NLR family, pyrin domain containing 1 [NLRP1] and NLRP3) with their PAMP or DAMP ligands.[136–138] Caspase-1 is thought to be the executioner caspase in inflammation, important in the synthesis of active IL-1β and IL-18 and thus pathogen defense. Hence, mice deficient in caspase-1 are more susceptible to bacterial infections and sepsis[139–141]; in contrast, mice deficient in caspase-12, which normally suppresses caspase-1 activity, show enhanced bacterial clearance and resistance to sepsis.[142] Parallel observations have been made in human patients possessing the full-length caspase-12 proenzyme—uniquely present in approximately 20% of people of African descent—which attenuates the innate immune response to endotoxin and is thought to be a risk factor for the development of severe sepsis.[143] Whereas caspase-1 is essential for host defense against

pathogens, its activity needs to be tightly controlled. Excessive caspase-1 activity and thus endotoxic shock was induced in mice given high doses of LPS, but mice deficient in caspase-1,[144] or ASC in another study,[145] were resistant to the lethal effects of LPS. Inflammasome activity has also been linked to the phenomenon of immunoparalysis. Thus, the expression of mRNA encoding the key inflammasome components ASC, NALP1, and caspase-1 was decreased in human monocytes during early septic shock, thought to reflect monocyte deactivation; furthermore, NALP1 mRNA abundance was linked to survival in patients with sepsis.[146] Very little is known about inflammasomes in the veterinary species, although inflammasome assembly mRNAs have been analyzed in the ovine jejunum[147] and induction of inflammasome genes in the spleen was documented in septic pigs infected with either *Haemophilus parasuis*[148] or *Streptococcus suis*.[149]

Pattern Recognition Receptors: Veterinary Species

After mapping of the gene sequences for human and murine PRRs, exploration of the genomic sequence of other species has allowed the identification of homologs of the majority of TLRs in dogs,[150–154] cats,[49,151,155,156] cattle,[38,50,51,62,157–168] sheep,[48,51,169–173] goats,[52,174,175] horses,[47,168,176–181] and pigs.[169,182–191] Other PRRs have also been identified in these species, although less is known about them than the TLRs (Table 3).

Toll-like receptors 1–10 have been identified in the bovine genome,[50,161] with numerous studies documenting their expression in tissues as varied as the endometrium,[38,163] cornea,[158] mammary gland,[167,192] skin,[51] and lung.[168] Whereas most of these studies have utilized PCR techniques to identify TLR expression,[38,51,158,163,167] an increasing number are employing flow cytometry[192,193] or immunohistochemistry.[168] TLR signaling in cattle is similar to that described in mice and humans (Fig 2)[162,192,194] and the PKR/elF2α pathway appears to be important in bovine viral diarrhea virus (BVDV) and rotavirus infections.[195,196] Comparatively little is known about the NLR family in cattle, although mRNA encoding NOD 1 and 2 has been identified in bovine mammary tissue.[197,198] Because of the financial implications of Johne's disease and the currently unconfirmed link between *Mycobacterium avium* ssp. *paratuberculosis* and Crohn's disease, a number of studies have examined the potential role of polymorphisms of candidate genes—including TLRs—in susceptibility to paratuberculosis.[199] Recently, TLRs 1–10 have also been cloned and sequenced in sheep[48] and goats.[174] Little is currently known about the implication of TLRs in sepsis of ruminants, although the pathways involved in triggering SIRS in sheep appear to be similar to those reported in other species.[169,200]

The impact of infectious agents upon commercial viability of pigs and the contribution of this species to human disease modelling has helped advance the characterization of porcine PRRs.[191] Thus, TLRs 1–10 have been cloned and sequenced[201–208]; surface and endosomal TLRs have been detected—both by PCR and immunohistochemistry—in a variety of porcine tissues and enhanced expression demonstrated in response to a number of infectious agents and PAMPs.[168,183,209–211] Members of the NLR family have also been identified[212–216] and current research suggests that porcine PRR signaling pathways are similar to those of other mammalian species.[169,217]

Various members of the TLR family have been identified in horses, including TLR 2, 3, 4, 5, and 9.[47,53] Whereas many of these have been characterized by PCR,[176–179] TLRs 4 and 9 have also been localized by immunohistochemistry and immunogold electron microscopy[168,179,181]—and a recent study demonstrated TLR 5 expression by equine neutrophils by flow cytometry.[218] Stimulation by PAMPs has increased gene expression of TLRs 2, 3, and 4 in vitro,[177] and TLR 9 *in vivo*,[181] whereas clinical studies have reported increased TLR 4 gene expression in both foals and adult horses with SIRS/sepsis, but no differences in expression between survivors and nonsurvivors.[219,220] Complementary studies have demonstrated increased plasma endothelin-1 concentrations and decreased long-term survival in horses with severe versus mild-to-moderate endotoxemia.[221] As for cattle and pigs, initial investigations have indicated that the downstream signaling pathways instigated by PAMP stimulation of equine TLRs 2–4 are similar to those identified in other species.[222] To date, there have been no published reports on members of the NLR family in the horse.

Less is currently known about PRRs in small animals.[53–55] Various PCR studies have confirmed the presence of members of the TLR family in both dogs, including TLRs 2, 4, 7, and 9,[61,151–154,223] and cats, including TLRs 1–9.[49,151,155,156] Comparative examination of the canine genome has also identified the presence of genes encoding members of the NLRP family, integral to inflammasome and thus caspase activation.[224] A number of canine TLRs have also been detected by immunohistochemical staining and flow cytometry.[168,209,225,226] The use of feline models for investigation of human diseases as diverse as type 2 diabetes mellitus and human immunodeficiency virus infection has yielded additional data on PRRs in cats, including the expression of functional TLRs by the endocrine pancreas[155] and modulation of TLR signaling by retroviral pathogens.[156,227] Enhanced expression of canine TLRs has been observed in clinical cases of osteoarthritis[60] and cystic endometrial hyperplasia/pyometra[61,226]; however, the majority of published research in dogs concerns the expression of PRRs in the intestinal tract, particularly in relation to inflammatory bowel disease (IBD).[150,223,228] Ongoing research is attempting to identify whether or not certain single nucleotide polymorphisms (SNPs) of PRRs can be related to the propensity for particular canine breeds to develop IBD and other immune-mediated diseases.[229–231] The analysis of SNPs in PRR-encoding genetic sequences is also an exciting field of research in large animals,[164,213,232] laying the foundation for the breeding of livestock with enhanced disease resistance and

the design of vaccines better able to target dendritic cells.[191,233] Finally, recent work has documented the expression of an ortholog of human "triggering receptor expressed on myeloid cells-1" (TREM-1) by canine neutrophils.[234] Expression of TREM-1 was upregulated by microbial agonists of TLR1/2, TLR2/6, and TLR4/MD2.[234] This receptor, which has shown promise as a biomarker of sepsis in humans, amplifies pro-inflammatory responses to microbial products.[59,235]

Inflammatory Mediators: Cytokines and Chemokines

PRR ligation triggers signaling cascades that culminate in the activation of NF-κB and AP-1 via MyD88 or TICAM1/TRIF (Fig 2).[236–238] NF-κB and AP-1 enter the nucleus and activate transcription sites for a variety of genes, including acute phase proteins, inducible nitric oxide synthase (iNOS), coagulation factors, and pro-inflammatory cytokines and chemokines, such as tumor necrosis factor (TNF)-α and ILs-1, 6, 8, and 12. The TICAM1/TRIF pathway results in the phosphorylation of interferon regulatory factors 3 and 7 (IRF3, IRF7), which likewise enter the nucleus and stimulate the transcription of genes encoding interferon (IFN)α, IFNβ, and other type 1 IFN-inducible genes.[236–238]

Serum concentrations of TNF-α correlate with death in certain types of human sepsis.[239,240] Studies of naturally occurring sepsis in veterinary patients have yielded similar results, although this pattern appears not to be universal: increased serum concentrations of TNF-α correlate with mortality in canine parvovirus and neonatal septicemia in cattle and horses, but not in septic cats.[11,241,242] TNF-α is predominantly produced by activated macrophages and T cells—but also by mast cells, B cells, NK cells, neutrophils, endothelial cells, myocytes, osteoblasts, and fibroblasts—as a 26 kDa precursor (pro-TNF) expressed on the plasma membrane; there it is cleaved by TNF-converting enzyme (TACE/ADAM17) to yield a 17 kDa soluble form, both the soluble and membrane-bound forms appearing to be active.[243] TNF-α exerts its effects by interaction with one of 2 receptors, TNF receptors 1 and 2 (TNFR1, TNFR2).[244,245] Activation of TNFR1 appears to mediate the proinflammatory and apoptotic pathways associated with inflammation, whereas TNFR2 plays a role in the promotion of tissue repair and angiogenesis.[245] However, the complexity of signaling networks operating in sepsis is underlined by the observation that NF-κB activity induces molecules that block apoptosis mediated by TNFR1, suggesting that integration of the various signals occurs *in vivo*.[246] Some "cooperation" between the 2 receptor types, particularly at low TNF-α concentrations, is also likely and stimulation of both TNFRs leads to further NF-κB and AP-1 release.[244,247]

Many of the classical features of inflammation can be attributed to the actions of TNF-α upon the endothelium, with increased production of iNOS and cyclo-oxygenase 2 (COX-2) leading to vasodilatation and local slowing of blood flow[248]; TNF-α also stimulates the expression of endothelial adhesion molecules such as E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1).[249,250] These 3 molecules lead to the tethering of leukocytes to the endothelial wall and their transmigration into the interstitium, accompanied by fluid and plasma macromolecules.[19] There is also evidence for the upregulation of TNF-α and other proinflammatory cytokines in a canine model of sepsis involving the intravenous infusion of low doses of LPS: increased serum concentrations of TNF-α, IL-1β, and IL-6[251–253] were accompanied in one of the studies by increased expression of pulmonary E-selectin and ICAM-1 and the influx of neutrophils—although whether the LPS induced the expression of the adhesion molecules directly or via induction of the proinflammatory cytokines, or both, remained unclear.[251] A similar phenomenon of up-regulation of proinflammatory cytokines, including TNF-α, has also been observed in cats[254] and horses[255–257] treated with intravenous LPS, as well as the transcription of neutrophil chemoattractants by equine endothelial cells stimulated by Th2 cytokines.[258] Furthermore, concentrations of TNF-α, IL-6, and endotoxin were all higher in the blood and peritoneal fluid of horses with colic than in a healthy control group.[259] The stimulation of feline whole blood with various PAMPs has also elicited the synthesis of proinflammatory cytokines, including TNF, IL-1β, and CXCL-8.[260] Plasma nitrite and nitrate, oxidation products of NO, have been examined in canine sepsis, in which they were present at higher concentrations than in dogs with SIRS alone.[261] An additional study showed that the inflammatory response to the intravenous administration of LPS, examined by measuring serum concentrations of TNF-α, IL-1 and IL-6, was mitigated in dogs fed a diet rich in fish oils, suggesting that diet may be an adjunct to conventional anti-inflammatory treatment.[262] In addition to the upregulation of iNOS, COX-2, and adhesion molecules, TNF-α also induces the expression of procoagulant proteins such as tissue factor (TF)—and down-regulates anti-coagulant factors such as thrombomodulin—leading to activation of the coagulation cascade.[263] Despite the important role for TNF-α in endothelial activation, experimental evidence suggests that direct stimulation of TLRs expressed by endothelial and vascular smooth muscle cells may provide an alternative pathway for the vascular dysfunction seen in sepsis.[264–266]

In addition to TNF-α, NF-κB activation results in the transcription of a number of proinflammatory interleukins, such as IL-1, IL-6, CXCL-8 (IL-8), and IL-12 (Fig 1). IL-1 acts in a synergistic manner with TNF-α in the "hyperacute" period after innate immune stimulation in sepsis.[267,268] Two proinflammatory forms of IL-1 (IL-1α and IL-1β) have been identified and induce the synthesis of adhesion molecules and cytokines by endothelial cells, encouraging leukocyte activation, endothelial tethering, and transmigration into the interstitium.[269,270] IL-1 also upregulates

iNOS and COX2 production, acts as the major endogenous pyrogen in fever, and increases corticosteroid release via hypothalamic effects.[269,271]

Another important proinflammatory cytokine in sepsis is IL-6: as well as stimulatory effects upon leukocyte activation and myeloid progenitor cell proliferation, IL-6 also triggers the acute phase response and is a powerful pyrogen.[272,273] Like TNF-α and IL-1, plasma concentrations of IL-6 are increased in sepsis and may be predictive of progression to multiple organ dysfunction and death.[17,274,275] Whereas enhanced gene expression of IL-6 correlates with death in septic foals, the opposite relationship appears to hold for serum IL-6 concentrations.[219,276] However, increased serum concentrations of IL-6 and IL-1β do correlate with death in reports of naturally occurring sepsis in dogs and cats.[11,13]

Serum concentrations of anti-inflammatory cytokines, most notably IL-10, also increase in sepsis.[277,278] This acts not only to inhibit the release of TNF-α, IL-1β, and IL-6 from monocytes and macrophages, but also to induce the production of IL-1 receptor antagonist protein (IRAP-1) and soluble TNFR, thus reducing circulating concentrations of these cytokines.[279] The critical role of IL-10 in mediating the balance between pro- and anti-inflammatory processes can be seen in experimental models: IL-10 knockout mice are profoundly susceptible to sepsis, whereas IL-10 administration prevents these consequences.[280] In clinical situations, however, the pattern appears to be more complex, with increased serum IL-10 concentrations associated with mortality in septic foals and humans,[281,282] but a lower prevalence of feline infectious peritonitis in cats infected with feline coronavirus.[283]

Two additional cytokines have recently been identified as being critical in sepsis. Macrophage migratory inhibitory factor (MIF), produced by the anterior pituitary gland, is present at increased concentrations in SIRS and sepsis[284,285]; serum concentrations have not only correlated with mortality, but inhibition of MIF appears to be protective.[286–288] Although the trigger for MIF release *in vivo* is unclear, it is thought to delay apoptosis of activated monocytes and macrophages, thus helping to perpetuate a proinflammatory state.[288] High mobility group box protein 1 (HMGB-1) is an endogenous protein involved in nuclear DNA stabilization.[289] However, after necrotic or apoptotic cell death, it is released into the circulation where it has direct pro-inflammatory actions.[290] In addition, HMGB-1 appears to potentiate the effect of certain PAMPs and DAMPs upon TLR-2, TLR-4, and the receptor for advanced glycation end products (RAGE).[291] HMGB-1 was initially reported as a late inflammatory mediator in sepsis,[292] although ongoing research has highlighted a number of roles in tissue repair and angiogenesis.[293] Circulating concentrations of HMGB-1 appear to correlate with mortality in canine SIRS patients,[294] but were unable to predict hospital mortality in 1 study of septic human patients.[295]

The Acute Phase Response

In addition to the release of cytokines and chemokines from activated immune cells, triggering of PRRs also bring about the release of large quantities of acute phase proteins (APPs) from hepatocytes; these proteins have a variety of functions designed to re-establish homeostasis, assisting in pathogen elimination and subduing inflammation.[296,297] The acute phase response is characterized by fever, neutrophilia, activation of the coagulation, and complement cascades (classical, alternative, and mannose-binding lectin pathways), serum iron and zinc binding, enhanced gluconeogenesis, increased muscle catabolism, and altered lipid metabolism.[297–299]

In general, 2 groups of APPs are recognized: type I, induced by IL-1α, IL-1β, and TNF-α, and type II, induced by IL-6.[298] As a consequence of the up-regulation of APP production, concentrations of other plasma proteins such as albumin, protein C, protein S, and antithrombin (collectively known as negative APPs) decrease.[299,300] Granulocyte colony-stimulating factor (G-CSF) released from monocytes is also an important component of the acute phase response, thought to mediate a protective role against bacterial infection by virtue of its impact on neutrophils.[301] A number of APPs have been characterized in veterinary species, including dogs, cats, cattle, sheep, horses, and chicken,[297,302] and they have been used as biomarkers of inflammation in both research and clinical arenas in these species.[303,304] Recent studies have identified adiponectin and insulin-like growth factor-1 as negative acute phase proteins in a canine model of endotoxemia.[305]

Much research has been conducted to determine whether or not serum levels of APPs are predictive of survival in sepsis, with procalcitonin and C-reactive protein (CRP) the focus of particular attention.[306] Plasma procalcitonin concentrations appear to correlate with bacteremia and organ dysfunction in human clinical studies.[306,307] Whereas it also appears to be a canine APP, it does not allow the discrimination of inflammatory or infectious from neoplastic disease when measured as a whole blood PCR assay[308]; however, extrathyroidal procalcitonin gene expression was documented in dogs with SIRS but not in healthy animals in a preliminary observational study.[309] The utility of CRP measurements has been assessed in a number of studies examining infectious,[310,311] inflammatory,[312–319] neoplastic,[320,321] and endocrine[322] diseases in the dog, including critically ill dogs[323]: in general, serum CRP concentrations provide a sensitive but nonspecific means of measuring inflammation, offering diagnostic and prognostic information in some disorders but not others. A recent study of SIRS and sepsis in dogs demonstrated a correlation between decreasing serum CRP concentration and recovery from disease, suggesting its use as a prognostic biomarker in this context.[324]

The Interaction of Inflammation and Coagulation in Sepsis

Despite not classically considered part of the innate immune response, the prevalence of coagulation disorders and disseminated intravascular coagulation (DIC) in sepsis underlines the intimate link between the inflammatory and coagulation pathways.[325,326] On a local scale, activation of coagulation may act defensively to impede the dispersal of pathogens and inflammatory mediators from the site of insult.[327] Clinical studies have supported experimental models demonstrating increased activation of coagulation, as well as downregulation of anticoagulant mechanisms and reduced fibrinolysis, in human SIRS and sepsis patients.[266,328] Similar findings have also been reported in dogs[329] and horses.[330] Whereas DIC[331] and pulmonary thromboembolism[332] have both been recorded in association with sepsis in cats, experimental models of endotoxin infusion in this species have yielded variable results,[333,334] a recent study failing to elicit any biologically significant alterations in coagulation parameters,[334] underlining the multifactorial pathogenesis of coagulopathies in clinical patients.

A detailed description of the complexities of the interaction between inflammation and coagulation is beyond the scope of this article; however, several comprehensive reviews of hemostasis in SIRS and sepsis in veterinary species have recently been published and an overview of key interactions is presented in Fig 4[335–337] In brief, key to the triggering of the coagulation pathway in sepsis is tissue factor (TF), which initiates coagulation via the contact activation (extrinsic) pathway. In health, lack of TF exposure within the vascular system and the presence of various circulating proteins—such as protein C, antithrombin, and tissue factor plasminogen inhibitor—modulate coagulation by the prevention of TF activation.[266,335] The expression of TF by monocytes or macrophages and tissue parenchymal cells is activated by various inflammatory cytokines, CRP, and PAMPs such as LPS[335,338]—a phenomenon that has also been documented in cats[339] and horses (earlier studies in this species citing "procoagulant activity" rather than TF per se).[340–342] Although findings differ between species, large numbers of TF-expressing microparticles have been identified in blood samples from septic human patients and may correlate with mortality.[343,344] These microparticles are released from a variety of activated or apoptotic cells—such as platelets, monocytes, erythrocytes, and endothelial cells—and their interaction with endothelial cells and platelets drives the coagulation pathway.[345–347] Although only an indirect measure, plasma von Willebrand factor concentrations were higher in septic dogs than those in healthy control animals, suggesting that endothelial cell activation also occurs in canine sepsis.[348] Interestingly, platelets enhanced endotoxin-induced equine monocyte TF activity in vitro[341]—although whether microparticles derived from platelets or other sources play a role in the pathogenesis of equine sepsis *in vivo* remains unknown. Additional work in an equine model of endotoxemia has shown that large volume resuscitation has no impact on coagulation parameters beyond the changes attributed to endotoxemia, providing useful additional data to inform the treatment of sepsis in this species.[349]

Whereas much research has been directed toward the inhibition of coagulation in sepsis, only activated protein C has shown any benefit in human clinical trials.[350,351] The antithrombotic and anti-inflammatory properties of recombinant human activated protein C led to recommendations for its use in severe sepsis in 2004[352] and 2008,[353] but a recent meta-analysis found no evidence in support of its administration in the treatment of severe sepsis or septic shock.[354] Indeed, there is still debate about its mechanism of action and, as yet, little experience of its use in veterinary medicine, despite encouraging pharmacological data in experimental models.[266,355,356]

In addition to the role of TF in initiating coagulation in the presence of inflammation, activated platelets and endothelial cells—as well as bacterial surfaces—also trigger the contact phase system, leading to the formation of kallikrein and bradykinin.[357,358] Bradykinin in turn enhances vasodilatation and increases vascular permeability, as well as reducing platelet function[359]; kallikrein accelerates fibrinolysis by conversion of plasminogen to plasmin and causes additional activation of Factor XII, leading to stimulation of the classical complement pathway.[360,361]

A final connection between coagulation and inflammation in sepsis has become apparent with the recent exploration of the role of "a disintegrin-like and metalloproteinase with thrombospondin type-I motifs-13" (ADAMTS-13). ADAMTS-13 is produced by the stellate (Ito) cells of the liver and acts to cleave ultra-large von Willebrand's Factor (vWF) multimers into smaller multimers.[362] These ultra-large vWF multimers are released from endothelial stores after inflammation and lead to platelet activation and aggregation; the ensuing microthrombi further compromise tissue blood flow, leading to additional propagation of the proinflammatory state.[363] Although not yet identified in clinical veterinary species, decreased plasma ADAMTS-13 activity is associated with a poor prognosis in human sepsis patients[364–366]; decreased activity is attributed to both a diminution of hepatic production and an increase in breakdown by plasma proteases.[366]

The Compensatory Anti-Inflammatory Response Syndrome and Cell Death

Ongoing investigation of the molecular mechanisms of the SIRS response, as well as the notable failure of therapeutic blockade of proinflammatory mediators, led to the realization that the mortality associated with sepsis could not be explained solely by an uncontrollable "cytokine storm".[367,368] This resulted in the concept of an opposing "compensatory anti-inflammatory response syndrome" (CARS), thought to be an adaptive response to the excessive proinflammatory process in SIRS and sepsis.[367,369] Whereas an appropriate balance is struck

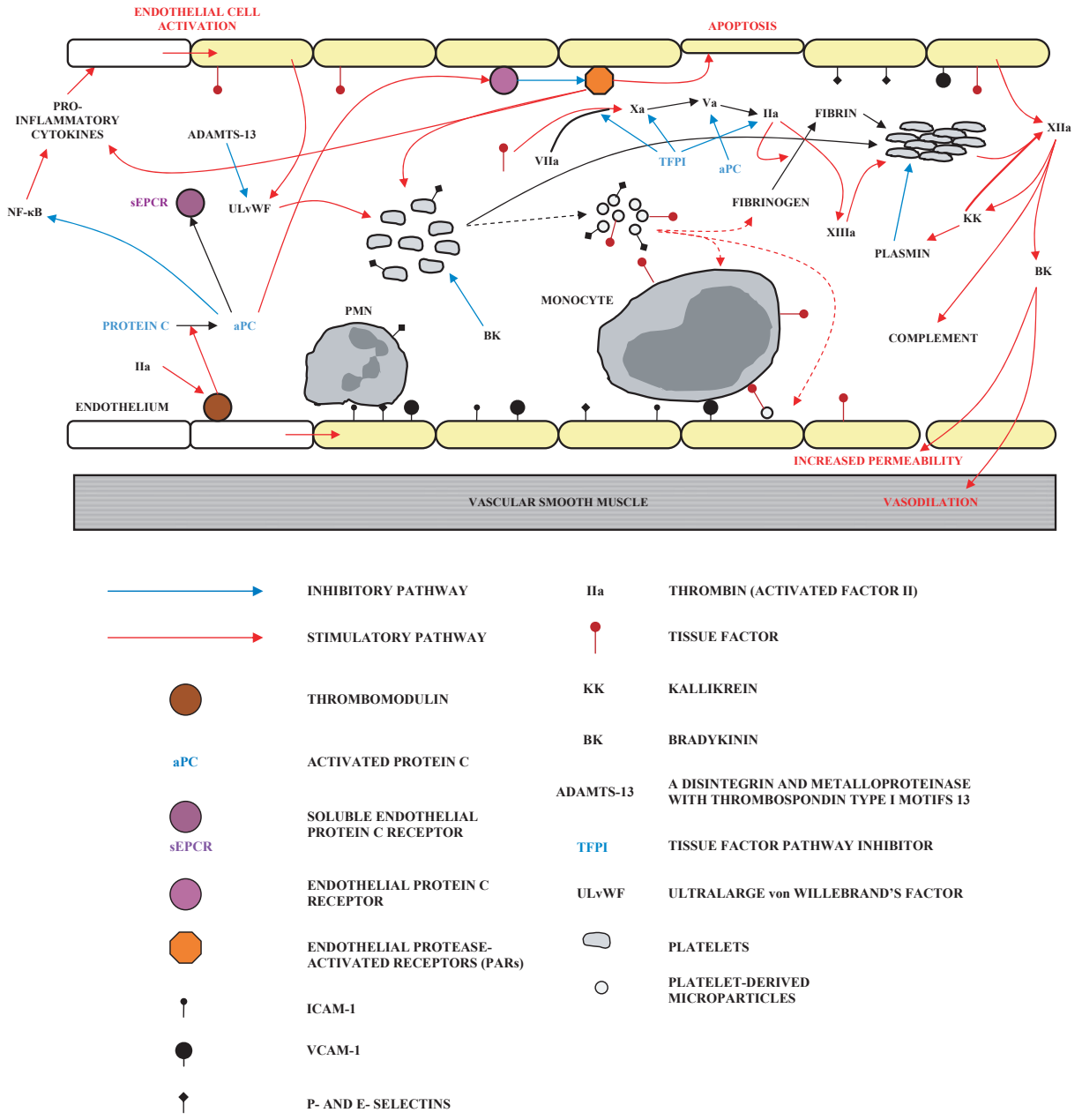

Fig 4. Points of interaction of the coagulation cascade and sepsis. In the absence of inflammation, various anticoagulant mechanisms exist to prevent activation of coagulation. Thrombomodulin and endothelial protein C receptor interact with thrombin to activate protein C (aPC), which in turn interacts with protein S to inactivate factor V. Endothelial cells also express tissue factor pathway inhibitor (TFPI) and antithrombin on their luminal surface and secrete tissue plasminogen activator (tPA). However, the majority of these anticoagulant factors are negative acute phase proteins and thus plasma concentrations falling during sepsis. Furthermore, proinflammatory cytokines released during sepsis activate endothelial cells, platelets and circulating white blood cells, which begin to express tissue factor (TF) and lose surface anticoagulant proteins. Microparticle formation is increased, together with expression of functional adhesion molecules on platelets, microparticles, monocytes and endothelial cells. Plasma antithrombin, aPC, protein S, and TFPI concentrations fall; ADAMTS-13 activity also drops, leading to greater persistence of ultralarge von Willebrand factor, which further enhances platelet and microparticle activation and adhesion. After triggering of the contact activation (TF) pathway, the interaction of microparticles with endothelial cells and platelets further drives the coagulation pathway. The presence of coagulation pathway components (TF-VIIa complex, Xa, Va, thrombin) and other circulating proteases activates endothelial protease-activated receptors (PARs), which propagate the inflammatory process by stimulating the further release of mediators (TNFα, IL-6 and CXCL-8), platelet activation and cell death. Triggering of the contact phase system activates factor XII (Hageman factor), leading to the formation of kallikrein (KK) and bradykinin (BK).

in the vast majority of instances of host-defense challenge, this is lost in sepsis—leading either to an uncontrolled proinflammatory reaction to infection, resulting in organ dysfunction, an undesirable compromise of the immune system permitting opportunistic infection (the "second hit"), or a combination of both.[367]

Rather than a sequential or compensatory change, as was initially proposed, SIRS and CARS appear to occur simultaneously, and act to balance the host's need to maintain defense while minimizing self-induced tissue damage.[370] Serum concentrations of both pro- and anti-inflammatory mediators increase early on in sepsis[370]; likewise, concentrations of a variety of both types of mediators (eg, IL-6, IRAP-1) are predictive of septic morbidity or mortality.[370–372] Thus the changes in cytokine profile are both dynamic and heterogeneous, indicating that prescriptive immunomodulatory therapies are unlikely to meet with success.[370,373] Furthermore, apoptosis of lymphocytes, hepatocytes, gastrointestinal epithelial cells and endothelial cells is increased, whereas that of neutrophils is decreased.[374,375] Neutrophil function is also altered: both the migration of neutrophils to infected tissues[376,377] and their antimicrobial function[378] is diminished; moreover, peritoneal neutrophils are a potential source of IL-10, suppressing inflammatory monocytes in a model of polymicrobial sepsis.[379] Although monocyte survival appears unaltered, sepsis results in the production of molecules such as IL-1 receptor associated kinase M (IRAK-M), MyD88 short variant (MyD88s), and A20-binding inhibitor of NF-κB activation3 (ABIN-3), which reduce activation of the NF-κB signaling pathway and therefore dampen the response to PAMP recognition.[380,381] Monocyte function is impaired in sepsis, with decreased expression of the MHC class II molecule human leukocyte antigen–DR (HLA-DR)[382,383] and decreased inflammasome expression.[146] Longitudinal observational studies in human patients indicate that a failure to regain >70% normal monocytic expression of HLA-DR is associated with an increased risk of secondary bacterial infection and decreased survival.[384] In addition to these changes in antigen-presenting function of monocytes, dendritic cells also undergo increased apoptosis in sepsis, further impairing the host's ability to respond to pathogens.[385]

Although apoptosis, or type 1 programmed cell death (PCD), is responsible for the majority of immune cell death in sepsis and is implicated in immunoparalysis, alternative pathways also play a role.[386,387] Autophagy is a cellular mechanism that primarily acts as a cytoplasmic "clean-up" process, as well as assisting in delivery of proteins to antigen presentation pathways; however, it may also mediate type II PCD and interact with apoptosis.[388,389] Much current interest has been directed toward the role of autophagy in trauma and sepsis, although whether it acts in a cytoprotective role or as a mechanism of PCD, or both, remains unclear.[386,390] A final mechanism of interest in the pathogenesis of sepsis is pyroptosis, a term used to describe the process of caspase-1-mediated PCD, which is distinct from death mediated by the apoptotic caspases 3, 6, and 8.[391] Although some question whether pyroptosis is truly a unique cell death mechanism, or simply a special case of apoptosis or necrosis (oncosis),[392] it is characterized by rapid plasma membrane rupture and release of proinflammatory intracellular contents, some of which may act as DAMPs.[393] One of the targets of caspase-1 during sepsis is the glycolytic pathway.[394]

A number of studies also suggest an important role for apoptosis in the pathogenesis of SIRS, sepsis, and infectious disease in veterinary species. Apoptotic cells were observed in the liver, kidney, thymus, stomach, and lymphocyte population of endotoxemic piglets[395] and the primary and secondary lymphoid organs of pigs infected with classical swine fever virus.[396] LPS and TNF-α both induced apoptosis of bovine glomerular endothelial cells, modeling a potential pathomechanism of acute renal failure in Gram-negative sepsis[397]; this phenomenon could be potently inhibited by glucocorticoids *in vitro*.[398] *Haemophilus somnus*, a Gram-negative pathogen of cattle that causes sepsis and vasculitis, induces caspases 3 and 8, and subsequent apoptosis, of endothelial cells in vitro[399]; furthermore, the bacterium stimulates platelets that are in turn able to induce endothelial cell apoptosis by a contact-dependent mechanism involving the activation of caspases 8 and 9 and the synthesis of reactive oxygen species.[400] The activity of matrix metalloproteinases 2 and 9 and the expression of phosphorylated Paxillin showed positive correlation with cardiomyocyte apoptosis in an ovine model of endotoxemic shock,[401] whereas apoptosis of peripheral blood mononuclear cells and splenocytes of sheep infected with bluetongue virus was thought to contribute to immunosuppression in this disease.[402] Ileal epithelial apoptosis was documented in one feline model of endotoxemia,[403] although a second demonstrated lymphocyte apoptosis in the spleen and Peyer's patches.[334] Apoptosis of T cells also contributes to the immunosuppression characteristic of feline immunodeficiency virus infection.[404] Finally, apoptosis of intestinal epithelial cells was observed in a canine model of sepsis induced by the intravenous infusion of *E. coli*.[405]

Regulatory T Cells and Sepsis

One of the key features of the adaptive immune system is its ability to generate antigen-specific receptors with an enormous diversity of specificities, some of which may recognize host-derived epitopes.[406,407] The majority of potentially autoaggressive thymocytes are deleted in the thymic medulla in a process called negative selection.[408,409] However, this process of central tolerance is imperfect and underlines the importance of a number of peripheral tolerance mechanisms, including clonal deletion, functional inactivation (anergy), and phenotypic skewing.[410–412] Although a subject of debate for many years, a population of Tregs is now known to play a major role in peripheral tolerance, complementing the preceding (intrinsic) mechanisms.[413–415] Little is currently known about tolerance mechanisms in veterinary species,[416] but Tregs have been identified in cats,[417–423] dogs,[424–433] pigs,[434–442] cows,[443–446] sheep,[447,448] and horses.[449–454]

Both naturally occurring and peripherally induced Tregs have been characterized—the former the product of a pathway of thymic differentiation called altered

negative selection and the latter the product of peripheral activation of conventional T cells in the context of an environment rich in transforming growth factor β (TGF-β) or IL-10.[455–457] Naturally occurring Tregs have been identified by their constitutive expression of the IL-2 receptor α chain (CD25) and Forkhead box P3 (FOXP3), a transcription factor that plays a pivotal role in both their ontogeny and peripheral function. FOXP3 acts to stabilize the Treg transcriptome by repressing a number of pro-inflammatory and growth-promoting genes—for example, *IL-2* and *IFNG*—while activating others encoding key molecules involved in Treg function—for example, *CTLA4* and *CD25*.[458,459]

Naturally occurring Tregs are known to interact with cells of both the innate and adaptive immune systems—including monocytes, macrophages, natural killer cells, neutrophils, mast cells, dendritic cells, and both T and B cells—generally mediating a suppressive function to prevent the development of autoaggressive responses and maintain the population of peripheral $CD4^+$ T cells, thus contributing to immune system homeostasis.[456,460,461] Tregs are also known to express a variety of TLRs, stimulation of which has augmented or abolished regulatory function in various studies.[462–465] The molecular mechanisms of immune suppression mediated by naturally occurring Tregs have not been fully elucidated, but involve cell contact-dependent interactions, induction of cell death, and secretion of immunosuppressive cytokines, including IL-10 and TGF-β.[416,456]

Various studies have documented increased proportions of Tregs in human sepsis during the phase of immunoparalysis,[466–469] but the role of this change remains unclear because depletion of Tregs in murine models of sepsis has yielded variable conclusions between models, either improving, enhancing, or bearing no influence on mortality.[470–472] Given the ability of Tregs to induce the alternative activation pathway of macrophages[473] and to inhibit the LPS-induced survival of monocytes through a proapoptotic mechanism involving the Fas/FasL pathway,[474] they may make a potentially significant contribution to immune system dysfunction in human septic patients,[468,469,475,476] although this is still a controversial area.[477]

Additional research is required to elucidate the role of Tregs in sepsis, as well as to identify their mechanisms of action in this context. Moreover, to the authors' knowledge no veterinary studies have interrogated Treg number or function in septic patients to date. Pharmacological manipulation of Treg activity continues to be explored experimentally and may eventually translate to clinical cases; however, therapeutic interventions to alter the resistance of immune cells to Treg suppression may prove an equally valid alternative approach.[478,479]

Conclusions

As is apparent from Figure 1, a broad concept of the immunopathological mechanisms underlying sepsis is now generally accepted. However, many of the molecular details are both complex and incompletely elucidated. Ongoing research has also indicated the existence of multiple redundant pathways within the innate immune response, potentially explaining the failure of many highly selective therapeutic interventions.[14,19]

A single "magic bullet" for the treatment of sepsis is highly unlikely to exist: an individually tailored set of therapies, based on point-of-care assessment of the immunopathological status of the patient, is the likely future—albeit a distant one.[373] Various human studies have resulted in the publication of consensus statements regarding the treatment of sepsis,[352,353] but current veterinary evidence to substantiate these interventions is thin on the ground. What has become evident is that outside the experimental laboratory, the heterogeneous nature of the septic patient population means that clinical trials must be carefully designed to obtain meaningful data.[480–482] Ongoing basic research into the immunopathology of sepsis in clinical veterinary species is equally important, to elucidate the underlying molecular mechanisms and thus direct clinical studies to those aspects of disease likely to benefit the greatest number of patients.

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