Genetic Variation in Innate Immunity Pathways and Their Potential Contribution to the SIRS/CARS Debate: Evidence from Human Studies and Animal Models

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Key Words

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Abstract

The lack of a causal and successful treatment for sepsis has led to a re-evaluation of the condition's pathophysiology. The failure of anti-inflammatory strategies has implied compensatory immunosuppression to play a central part in fatal clinical cases. While searching for novel therapeutic strategies, the question arose whether pro-inflammation (systemic inflammatory response syndrome, SIRS) or anti-inflammation (compensatory anti-inflammatory response syndrome, CARS) are dominant in sepsis, and may be counteracted by therapeutic measures. Here we ask whether in a given organism – man or mouse – the lack of any functional protein involved in this cascade may help in understanding the events. In humans, genetic variations exist, and some of them have functional consequences altering the inflammatory response to pathogens. In mice, knockout animals were created, which may assist us in understanding the SIRS/CARS cascade. Here we summarize data on genetic variations in the TLR- and cytokine system and their influence on course of infectious diseases and sepsis. In addition, we summarize

animal experiments and conclude that both cascades may be needed for containing infection. Imbalances in both the pro- and anti-inflammatory system may be harmful. Thus, interventional strategies have to be introduced carefully, and in the future genetic profiling may be needed in order to tailor therapies in the best way.

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Introduction

Systemic inflammation is a consequence of a variety of insults that are potentially damaging to any organism in nature. Its sequela of events leads to a uniform syndrome called systemic inflammatory response syndrome (SIRS) [1]. This syndrome was introduced into medical terminology in the early 1990s and has since had widespread acceptance as a model to describe particularly sepsis pathophysiology [2]. The initial steps in the etiology of this syndrome are largely due to inflammatory mediators induced by the innate immune response [3, 4]. The adequate timing and the diligent control of this response is thought to be decisive for the outcome of affected individuals. Although the pro-inflammatory part of this response is crucial for the initial success of the immune system, it may also cause harm. So the resolution of the

pro-inflammatory signals appears to be equally important for survival. If this counter-regulatory response comes at the right time, over-inflammation can be avoided and termination of the inflammatory response leads to complete restitution of the host. However, if the control mechanisms mediated by anti-inflammatory mediators are too pronounced or prolonged, anti-inflammation leaves the host in an immunosuppressive state where secondary infections might occur at the worst conceivable time [5]. This syndrome has been termed 'compensatory anti-inflammatory response syndrome' (CARS) [6].

The regulation of these pathways is influenced by a broad number of host proteins, either cellular receptors and signaling molecules, or soluble factors released by host cells locally or systemically. These factors include cytokines, chemokines and other regulatory proteins secreted by immune cells and others. The course and extent of both SIRS and CARS are quite variable among individuals, which may be caused by variations of the underlying genes leading to interindividual differences in responses of the patients [7]. Since many recent studies have shown that the host response is influenced by genetic variation of the individual, it is reasonable to try to explain the difference in the response to an insult/infection to a certain extent by these variations [8, 9].

Genetic variations of individuals can either be in intronic sequences of the genes involved or within the coding regions. Due to space limitations we will focus in this review mainly on coding mutations, although recent research shows that epigenetic mechanisms encoded in intronic sequences may constitute another important area of genetically determined individual differences. Some of the coding mutations are 'silent' and do not lead to an amino acid change and a subsequent change of protein function. The ones leading to amino acid changes may influence protein structure, functions, and stability and thus are most likely to have biological consequences. The non-coding mutations found in promoter regions can lead to a change of protein levels particularly during transcriptional activation of genes as frequently found during the SIRS/CARS cascade.

Functionally relevant single-nucleotide polymorphisms (SNPs) may eventually help in deciphering the complex phenomenon of SIRS/CARS and its relation to clinical outcome. First, this 'experiment of nature' may help us in identifying key elements of this cascade of events and relate them to the clinical course of disease, as genetic variations may influence outcome. Second, a lack of genetic variation may point to a central role of a protein, as major variations leading to a lack of function may

not have been compatible with life and thus may have been eliminated over time. In this short review we thus try to summarize results from genetic trials and will furthermore relate them to results obtained from knockout mice. Here a chosen gene is lacking completely, and an altered response during experimental sepsis/SIRS induction may point to a central role of the gene/protein of interest.

To date, however, there is no established animal model for SIRS and CARS that can be experimentally used to simulate genetic influence by employing knock-out-animals. This review therefore will focus on examples for animal models that are influencing either pro- or anti-inflammatory pathways in the context of inflammation triggered by bacterial infections. A similar approach using data employing several SNPs in the pro- or anti-inflammatory portions of the innate immune system will be used to describe potential influence in patients suffering from major trauma, sepsis or major surgery which are the most common triggers for systemic inflammation.

Influence of Genetic Variations of the Inflammatory Cascade on the Outcome of SIRS

SIRS is considered to be a result of the release of cytokines, where the magnitude of this release is related to the intensity of the response [2]. As a consequence, genetic variants that result in an altered cytokine response are likely to influence the extent of SIRS. Variants involved in the first critical steps of the innate immune response are thought to influence sensing of danger, initiation of intracellular pathways and release of cytokines via the induction of transcription factors, such as NF- κ B. Within the space limitations of this review we will focus on genetic variations within the Toll-like receptor (TLR) pathway initiating the inflammatory response, signaling elements of this pathway, and the pro-inflammatory cytokines. Table 1 lists a selection of common variants of pro- and anti-inflammatory elements involved in the systemic inflammatory response.

SNPs in TLRs and the SIRS/CARS Cascade

The family of TLRs has only been discovered over the last 12 years. The first receptors identified were TLR4 and -2, cell-surface bound receptors recognizing, as we know now, lipopolysaccharide (LPS) of Gram-negative bacteria, and lipopeptides and other ligands originating from bacteria, fungi, and parasites (reviewed in many papers, such as [10]). Intracellular TLRs recognizing microbial

Table 1. Single-nucleotide polymorphisms in innate immune pathways in humans influencing pro-inflammatory cytokine response

Molecule	Genetic variant	Effect on cytokine	Function	Clinical effect	Ref.
<i>Sensing</i>					
TLR2	Arg753Thr	Less TNF and IFN upon stimulation; hyporesponsive.	Loss	Association with Gram-positive infection.	[24, 30, 33]
TLR4	Asp299Gly, Thr399Ile	Less TNF; hyporesponsive.	Loss	Association with <i>Candida</i> BSI; sepsis; Gram-negative septic shock.	[13–17]
CD14	–159 or –260	Higher levels of sCD-14.	Gain	Tendency towards higher mortality; conflicting results.	[15, 16, 23–26]
<i>Signaling</i>					
TIRAP/Mal	Ser180Leu, D96N	No influence.	Loss	Protection if heterozygous; higher risk for homozygous patients; Ser180Leu; rare mutation D96N.	[39, 40, 107]
<i>Cytokines</i>					
TNF	Multiple promoter variants	Higher levels of TNF.	Gain	Association not clear. Early studies suggest higher risk when homozygous.	[44–49]
IL-6	–174, –572, –6331	Contrary results (see text); lower IL-6 concentration in the CC allele in sepsis; higher following CABG.	Mixed	C-allele confers increased risk of shock; GG allele showed reduced mortality.	[55–60]
IL-1 β	–511, C3953T	No significant influence; see IL-1RA, below.	None	Higher mortality in homozygous carriers with meningococcal sepsis.	[15, 50, 51]
TNF-R	TNFRSF 1A, TNFRSF 1B	No influence.	None	Association not clear.	[48]
IL-10	–592 and –1082	Lower IL-10 levels.	Loss	For –592 and –1092 higher mortality; association for –1082 G-allele with SIRS and lower IL-10 levels.	[62–64]
IL-1RA	Intron2, IL-1RN* –2018	Lower IL-1RA serum levels.	Loss	Higher mortality in homozygous carriers; high mortality in meningitis associated with IL-1 –511.	[52, 53]
TGF- β and pathway	Superfamily genes SNPs	Higher TGF- β .	Gain?	Risk association with bacteraemia in TGF- β superfamily variants; higher TGF- β levels (tumor patients).	[68, 69]
CABG = Coronary artery bypass grafting; Mal = MyD88 adaptor like; TIRAP = TIR-domain-containing adaptor protein.					

nucleic acids of mainly viral origin have been identified and functionally characterized only lately, and, furthermore, genetic variations within these receptors are absolutely rare [11]. Studies addressing the potential role of genetic variations in TLRs thus mainly involved TLR4 and -2.

TLR4

Within the LPS receptor TLR4, two SNPs occur quite frequently in patients in the West, which lead to amino acid exchanges at position 299 and 399. These two SNPs, Asp299Gly and Thr399Ile, among Caucasian individuals are usually co-segregating and heterozygous carrier frequency is approximately 8–12% [12]. After discovery of this SNP around 10 years ago, it was found that carriers had a reduced airway reactivity upon inhalation with LPS. Furthermore, when epithelial cells were stimulated ex vivo, it appeared as if this genotype would lead to a loss-of-function phenotype [13]. Although some studies

showed relevance of the Asp299Gly/Thr399Ile SNP in Gram-negative infections, others were not able to confirm this [14–16]. However, the Asp299Gly (alone) haplotype, which is very rare among Caucasians, was found to be relevant with regard to sepsis severity and susceptibility to Gram-negative infections [17].

Interestingly, in vitro stimulation of blood cells failed to show any functional relevance of the Asp299Gly/Thr399Ile SNP [18]. In contrast, the Asp299Gly (alone) haplotype, the predominant variation in Africa, leads to an increased inflammatory response after ex vivo cell stimulation [19]. The authors compared the worldwide distribution of this SNP and concluded that it may provide an advantage for fighting malaria, and thus may have been selected in malaria-endemic regions while it was lost in other parts of the world. Taken together with the relatively small study by Lorenz et al. [17], it can be concluded that a rare gain-of-function mutation (Asp299Gly alone), leading to an increased inflammatory re-

sponsiveness of the cells, increases the risk for sepsis and Gram-negative infections.

Genetic variation of TLR4 also may exhibit an influence on the risk for bloodstream infection with *Candida*, but there is no clear evidence that TLR4 is involved in the sensing of pathogenic fungi [20]. There are molecules associated with TLR4 sensing including MD-2, CD14 and LPS binding protein (LBP). In all these molecules mutations or SNPs have been described that lead to a decreased inflammatory response to LPS [21–23]. Variations regarding CD14 have been shown to increase the risk of sepsis [24, 25]. Other studies, however, failed to confirm these results in patients with Gram-negative bacteremia [15]. In carriers of the polymorphic TT allele, levels of sCD-14 were higher and potentially modulate the inflammatory response [26]. In addition, it cannot be concluded whether a lack of LBP function clearly leads to a hypo-inflammatory state as LBP has been shown to also exhibit an LPS-inhibitory role when present in high concentrations [27, 28]. Our own results point to a role of a novel functionally relevant LBP SNP for the course and survival rates of sepsis [Eckert et al., 2010, submitted].

TLR2 Receptor Complex

A functionally relevant loss-of-function SNP that in European populations occurs as frequently as 10% in its heterozygous form has been described for TLR2 [29]. This mutant involves the exchange of arginine at position 753 with glutamine (Arg753Gln) and is located within the TIR domain of TLR2 [30]. After stimulation of immune cells with the mutated form of TLR2, fewer cytokines are released as compared to the wild-type form [31]. Interestingly, this SNP is completely absent in Africa, which has been related to the central role of the TLR2 complex in sensing *Plasmodium* [32]. *Staphylococcus aureus*, the most prominent Gram-positive bacterium playing a major role in sepsis, has been shown to induce inflammation through TLR2. It has been proposed that the Arg753Gln TLR2 SNP predisposes individuals to *S. aureus* infections; however, a larger study concluded that this is not the case [30, 33]. Another promoter SNP of TLR2 at position –16933 was associated with increased prevalence of sepsis and with Gram-positive bacteremia [24]. In conclusion, a loss-of-function mutation leading to a decreased inflammatory response in its heterozygous form most likely does not have a profound effect on the SIRS/CARS cascade, as the results are controversial.

Recently, we and others have described that TLR2 forms a heterodimeric receptor complex with either TLR1

or –6 [29]. Furthermore, TLR-10 may be another part of this complex and the TLR-1/6/10 genes are highly related and linked via haplotype blocks. Functionally relevant SNPs in TLR1 have been described, and they do play a role for the course of infectious diseases [34, 35]. Interestingly, these examples show that a loss-of-function mutation leading to a decreased cytokine response protects from disease. However, as these trials focused on leprosy, which is an extremely slow infectious disease, it is currently unclear whether these findings may have any implication for an acute inflammatory process, such as the SIRS/CARS reaction complex.

Genetic Variations in TLR Signaling Molecules

While the extracellular pattern recognition receptors display some redundancy, and some loss-of-function genetic variation may be tolerable by the host, the central signaling molecules such as MyD88 and IRAK4 display very little genetic variation. Some rare mutations, however, have been described leading to severe infectious complications during childhood [36, 37]. Carriers of defects in MyD88 and IRAK4 display a complete loss of the inflammatory response, and are highly susceptible to pyogenic infection during childhood. Affected individuals have a very high risk of dying from infection; however, individuals who survive past the age of 12 are clinically normal, which is most likely due to the by then matured acquired immune system [38]. This indicates a central role for these signaling molecules in innate immunity and corresponds to the animal models mentioned below. Notably no common polymorphisms have been described, probably indicating a strong evolutionary disadvantage if this loss-of-function variant exists. Both the IRAK4 and MyD88 mutations are loss-of-function, leading to infections that are afebrile. Thus, in this rare setting, a lack of (pro-)inflammation clearly leads to a worsened outcome during infection. However, this adverse effect may be due to the complete loss of bacterial recognition rather than due to a hypo-inflammatory state itself.

Another intracellular signal transducer of the TLR system was termed Mal/TIRAP, and a functionally relevant genetic variation has been described that in its heterozygous form is quite frequent, and may be associated with sepsis [39]. In a study involving patients with mycobacteriosis, functional assays showed an influence of this SNP on the cytokine release of host cells [40]. Furthermore, heterozygosity conferred protection against infection whereas homozygous carriers had worse outcome in patients with bacterial infections. Interestingly, the fre-

quency of the TIRAP/Mal SNP also differs greatly worldwide, which may point towards a selective pressure in areas of endemic diseases, which are mainly of infectious nature [41, 42].

Pro-Inflammatory Cytokines

Cytokines play a major role both in innate immunity and in the organization of an orchestrated adaptive immune response to local and generalized infections. In general, a distinction between pro- and anti-inflammatory cytokines exists. However, several cytokines have both properties, depending either on the time of release or the target, which is true for example for IL-6 [43]. A number of variations in cytokine genes have been reported in gene association studies.

Tumor Necrosis Factor

TNF is a prototypical pro-inflammatory cytokine. Three promoter polymorphisms in the TNF gene have been described: -238, -308 and -376. The -308 SNP has been studied especially extensively and an association with sepsis severity and outcome after different inflammatory insults has been found repeatedly, with a tendency towards increasing levels of TNF and therefore a stronger inflammatory response [44–46]. This is considered a factor contributing to sepsis severity and finally results in increased mortality [47]. However, these results have not been confirmed in recent studies [15, 48]. Most recently a meta-analysis found the -308 SNP resulting in a gain-of-function phenotype with increased release of TNF into the serum to be associated with increased risk for sepsis but not mortality [49].

The Interleukin-1 Family

Another key pro-inflammatory cytokine is IL-1 (isoforms α and β). An IL-1 β polymorphism was described for intron 6 (variable number tandem repeats, 46 base pairs), but this variation failed to show any association with sepsis [50]. However, an IL-1 β polymorphism in the promoter region (-511) was associated with worse outcome in meningococcal disease [51]. IL-1 receptor antagonist (IL-1RA) is involved in the control of the IL-1 activity by competitively inhibiting IL-1 receptors. Thus, this mediator is thought to act as an anti-inflammatory one. Homozygous carriers of a common SNP in this gene show lower IL-1RA levels in vitro and have a worse outcome during sepsis [52]. These individuals with lower IL-1RA levels exhibit a more pronounced inflammation, and this apparently leads to more severe sepsis. However, elevated levels of IL-1RA were also reported in stimulated

PBMCs from healthy subjects when carrying the particular genotype [53].

Recently in caspase-12 (most likely involved in IL-1 maturation and apoptosis), an SNP has been shown to confer an increased risk for septic shock in patients of African American descent [54]. Patients carrying this variant were found to produce a full-length caspase pro-enzyme (Csp12-L), which leads to impaired cytokine release upon in vitro stimulation. In contrast, in this small study patients producing a truncated protein (Csp12-S), the predominant form found in the West, were found to be protected from sepsis.

Interleukin-6

IL-6 is also regarded as a pro-inflammatory cytokine, although it exerts its function on a broader base also owing to anti-inflammatory properties by activating inhibitory mechanisms of the acute-phase response. In patients with SIRS, mortality rates were increased in relation to existing IL-6 haplotypes [55]. For the IL-6 promoter polymorphism (-174) evidence is accumulating that presence of the C allele is associated with increased risk of septic shock [56, 57]. Overall, data concerning plasma levels of IL-6 and the IL-6 (C-174/CC) allele are not conclusive [58, 59]. Of note, IL-6 levels following cardiac surgery are lower in carriers of the C allele whereas patients with septic shock display higher values compared to wild-type patients [60]. Another study that compared haplotypes containing the -174, -572 and -6331 SNPs found a stronger association with the latter SNP, questioning the functional importance of the -174 SNP. So, although some evidence is accumulating that genetic variations may influence the SIRS/CARS cascade, it is unclear whether cytokine levels are enhanced or diminished in the subgroups.

Anti-Inflammatory Cytokines

IL-10, the most prominent member of the anti-inflammatory cytokines, inhibits the synthesis of a number of cytokines, including IFN- γ , IL-2, IL-3, TNF and GM-CSF, produced by activated macrophages and by helper T cells. Other anti-inflammatory factors involved are IL-4, IL-13, TGF- β and, as mentioned above, IL-1RA [61]. Compelling evidence that these factors exclusively lead to an immunosuppressive state, however, is sparse. Thus the regulation of a broad array of immune functions by down-regulating pro-inflammatory cytokines as well as reducing monocyte and T cell activity might lead to immunosuppression. However, the most adequate extent of this anti-inflammation in the context of dampening of the inflammatory response has not yet been defined.

IL-10 is considered an important anti-inflammatory cytokine [61]. Stimulation of whole blood with LPS resulted in a broad variation of IL-10 release, which was associated with certain haplotypes, namely the IL10.R3/G7 haplotype containing two promoter microsatellites which had the lowest median IL-10 secretion [62]. Association studies of IL-10 SNPs (-592) and (-1082) showed a higher risk for sepsis for carriers of the respective mutations [63, 64]. However, the haplotype analysis of the highly variable 10.G micro satellite region of the gene promoter showed no influence on severity of sepsis [65].

Additional factors with known anti-inflammatory effects include IL-4, IL-13 and TGF- β . With regard to infections in humans, a common haplotype of the IL4 promoter (-1098T/-589C/-33C) was over-represented in patients with chronic disseminated candidiasis, whereas another common haplotype (-1098T/-589T/-33T) appeared to be protective [66]. These data were derived from a patient cohort with acute leukemia and no data on cytokine levels are available. Another study revealed altered IL-4 levels in patients with paracoccidioidomycosis [67]. IL-13 is a cytokine that, among other functions, inhibits inflammatory cytokine production. Numerous SNPs have been identified, but data on acute inflammatory states are not available.

TGF- β 1 is believed to be important in regulation of the immune system by blocking activation of lymphocytes and monocytes. When examining members of the TGF- β superfamily (IGF1R and genes of the TGF- β /BMP pathway like BMP6, TGFBR3, BMPRI1A, SMAD6 and SMAD3), genetic variation was associated with an increased risk of bacteremia in children with sickle-cell anemia [68]. Other variants in the TGF- β gene itself resulted in altered serum levels, but this study did not examine an inflammatory state but rather cancer risk [69]. Overall, results are not conclusive for susceptibility and course of sepsis.

Lessons Learned from Murine Knockout Models in the Context of SIRS and Sepsis

Although it is known that small rodents behave quite differently from mammals during the inflammatory response, in recent decades the technology of knocking out genes has become important in deciphering the function of a given gene/protein. It has been particularly important in the field of innate immunity, where the functions of a large number of receptors have been identified by generating knockout mice. Animal models in this

context are usually used to study the consequence of knocked-out genes in critical pathways for the inflammatory response. SIRS has not been defined for animals, so usually other features of systemic inflammation are used in these circumstances, such as release of cytokines, fever and weight change of the animals [70]. Studies involving inflammatory responses have employed almost all members of the above-mentioned pathways, with special focus on LPS response since it is used routinely to simulate inflammatory states. In this short review it is impossible to list all murine sepsis models; however, a selection representing key innate immunity receptor cascades is summarized in table 2.

Murine Models for SIRS

To date, a large number of studies exist using the effects of genetic knockout on the recognition of danger signals, which are the cornerstone of innate immune activation. So-called pattern-associated molecular patterns (PAMPs) or danger-associated molecular patterns are principal triggers for the development of systemic inflammation. Receptors for these danger signals are called pattern recognition receptors. TLRs are responsible for the detection of a large number of patterns, including endotoxin and others which are reviewed in detail in [71].

TLR Knockout Animals

After discovery of the first human TLRs, knockout mice became available quickly and were mainly bred in the Akira lab in Japan. For defining the ligand of a given TLR, usually a PAMP as mentioned above, knockout mice were stimulated with the respective microbial ligand and the cytokine response was compared to wild-type animals. So, for example, the TLR4 mouse lacked LPS responsiveness, the TLR2 did not induce inflammation following lipopeptide stimulation, etc. If lethal concentrations of the respective ligands were used, knockout mice were protected as the PAMP-induced 'cytokine-storm' was not observed. However, as we know now, this situation does not mimic the clinical situation of sepsis, instead infection models have to be looked at.

For TLR4, the LPS receptor, it was found that a murine strain termed C3H/HeJ found to be unresponsive to LPS challenge many years ago, contained a TLR4 mutation [72]. This strain had also been used for infection experiments and here it was found that this strain was more susceptible to, for example, a *Salmonella* infection [73]. So, in this mouse model a lack of inflammatory mediators was paralleled by a higher susceptibility for infection.

Table 2. Effect of selected knocked-out genes in mice located in innate immune pathways

Knockout	Model	Outcome effect	Ref.
Sensing			
TLR2	Peptidoglycan, lipopeptide	Less cytokine production.	[83]
	Live bacteria: <i>S. aureus</i>	Highly susceptible to infection; high mortality.	[78]
	Live bacteria: Group B Streptococci	Low dose: strongly pronounced spread of bacteria; high dose: attenuated cytokine response.	[79]
TLR4	LPS	Almost completely abrogated response.	[72]
	<i>Candida</i> infection	Stronger outgrowth of <i>Candida</i> , lower chemokine levels and impaired neutrophil recruitment.	[74]
	Live bacteria: <i>Klebsiella</i> , <i>Pseudomonas</i> , <i>Legionella</i> , <i>Haemophilus</i> etc.	Many experiments show specific response patterns towards single strains and types of pathogens. Animal models used to decipher specific roles of receptors. Mortality data show in part worse and in part better survival.	miscellaneous
CD14	LPS	Resistance to shock; reduced dissemination of Gram-negative bacteria.	[77]
Signaling			
Myd88	Peritonitis, polymicrobial	Better survival of animals.	[81]
	Live bacteria: Group B Streptococci	Low dose: strongly pronounced spread of bacteria; high dose: attenuated cytokine response.	[79]
	Live bacteria: <i>S. aureus</i>	Highly susceptible to infection; no survivors.	[78]
TIRAP/Mal	LPS	Impaired cytokine production.	[83]
	Live bacteria: Gram-negative	Worse survival.	[85, 86]
Cytokines			
TNF	LPS	Highly resistant to LPS; no TNF in serum, but normal IL-1 levels.	[87]
	Live bacteria: <i>L. monocytogenes</i>	No survival in infection.	
TNF-R	LPS	Resistant to LPS.	[88]
	Live bacteria: <i>L. monocytogenes</i>	High mortality.	
IL-1 β	Turpentine, LPS, <i>L. monocytogenes</i>	Normal response to LPS and live bacteria; protection against turpentine-induced SIRS.	[90]
ICE	LPS	Resistance to LPS-induced shock.	[91]
IL-1R type I	Turpentine, <i>L. monocytogenes</i> , <i>S. aureus</i>	Resistance to turpentine-induced shock; higher susceptibility to <i>L. monocytogenes</i> and <i>S. aureus</i> .	[92, 93]
IL-6	LPS, turpentine	Reduced release of acute phase response proteins.	[70]
	Live bacteria: <i>L. monocytogenes</i> , <i>S. pneumoniae</i>	Reduced release of acute phase response proteins; higher cytokine levels (pro- and anti-inflammatory).	[94, 95]
IL-10	<i>E. coli</i> -induced peritonitis	Increased mortality and organ failure despite reduced bacteremia.	[96]
	CLP plus rescue-surgery	Increased irreversible septic shock.	[97]
TGF- β	Spontaneous model	Death due to (sterile) hyperinflammation caused by upregulated TLR4.	[98, 99]
Mal = MyD88 adaptor like; TIRAP = TIR-domain-containing adaptor protein.			

Also in a model of *Candida* infection, TLR4-deficient mice had a worse outcome, although it is currently unclear how fungal infections are linked to TLR4 [74]. As mentioned above, the TLR4 complex sensing LPS consists of several accessory proteins, such as LBP and CD14. For

LBP it also was found early that while it enhances cytokine induction, and thus prevents septic shock induced by LPS, it is required for successfully combating infections: LBP knockout mice did much worse in a model of *Salmonella* infection [75, 76]. Mice lacking CD14, inter-

estingly were protected against both LPS- and live bacteria-induced sepsis [77].

Similar observations were made for TLR2 when the knockout mice became available: absence of TLR2 prevented the induction of inflammatory mediators by the TLR2 ligand lipopeptide. At the same time, these mice were highly susceptible to infection by Gram-positive bacteria such as *S. aureus* or group B Streptococci [78, 79]. This pattern holds true for several molecules of the innate immune system: a lack of immediate cytokine response to microbial trigger is paralleled by a worsened outcome in experimental infection models. Of course, experimental models always have limitations, and, as mentioned, mice and humans in particular are not easily compared in their innate immune reaction pattern. Still, these results point to the fact that a pro-inflammatory response is needed for combating infection and survival.

Mice Lacking TLR Signaling Molecules

The next critical step in the development of the inflammatory response after recognition and receptor binding of microbial products is signal transduction. There are several adaptor molecules involved in this process. A pivotal example is MyD88, one of two central adaptor proteins together with Mal/TIRAP. When used in an endotoxin model of inflammation it could be shown that disruption of MyD88 leads overall to an almost completely blunted cytokine response with a positive effect on survival [80]. When employing a model mimicking abdominal sepsis in mice the inflammatory response was also diminished with a survival advantage in MyD88 knockout mice. The same study could show that neutrophil invasion and bacterial clearing were not different compared to wild-type mice. Additionally, it was shown that distant responses in liver and lung were attenuated, possibly indicating a reduced effect of systemic cytokines [81].

In a different study, mice lacking MyD88 also displayed reduced cytokine levels but exhibited worse survival in an infection model using *S. aureus* [78]. So it appears that mice lacking a critical intracellular adaptor for TLRs show aberrant cytokine responses, which are beneficial in a scenario mimicking inflammation. However, this diminished response clearly affects their ability to fight infection in either a beneficial or disadvantageous way. When examining apoptosis of lymphocytes, which is thought to be a main contributor to 'immuno-paralysis' in late sepsis, disruption of MyD88 was associated with lesser apoptosis. However survival was worse in this mouse cecal-ligation-puncture (CLP) model, which

mimics human polymicrobial sepsis [82]. The pro-inflammatory response via MyD88 apparently can be strongly influenced by genetic disruption, which corresponds well to the results obtained with children having a rare defect in MyD88 as mentioned above [37].

Another central TLR signaling molecule is Mal/TIRAP, a second adaptor protein for the activation of NF- κ B. When this intracellular pathway is knocked out, cytokine response is defective [83]. However, in Mal/TIRAP signaling alternative intracellular routes may prevail and the response is switched towards the TRIF/TRAM-dependent IRF pathway [84]. So disruption of this adaptor can potentially switch response towards either diminished cytokine levels or to a predominantly interferon- γ -based response. As a consequence, genetic alteration of important mediating steps in the innate immune response lead to altered extent of immune responses that might interfere with the extent of anti-inflammation. In models with bacterial infection caused by *Escherichia coli* or *Klebsiella pneumoniae* it was shown that mice lacking Mal/TIRAP had higher mortality paralleled by a reduced cytokine response [85, 86]. Of note, no difference in outcome was found when comparing Mal/TIRAP knockout mice to control animals in a model of *P. aeruginosa* infection.

Pro-Inflammatory Cytokine Knockout Animals

Within the large number of pro-inflammatory cytokines, here TNF and IL-1 were chosen as key mediators of the SIRS reaction cascade. TNF as the prototypical pro-inflammatory cytokine has many implications in innate immune function. TNF knockout mice exhibited reduced overall cytokine response upon stimulation with LPS [87]. TNF acts as an early mediator in the cytokine response to inflammatory stimuli and produces early hypothermia or other symptoms in these animals. This is in contrast to the reaction towards live pathogens like *Listeria* and *Mycobacteria* [87]. Obviously, for the development of an adequate bacterial elimination the functions of TNF in the adaptive immune system are relevant. This indicates the importance of influencing SIRS in the long-term immunosuppression thought to be associated with prolonged inflammatory states. Another possible experimental disruption of the TNF signaling pathway is achieved by altering TNF receptors. Mice lacking both TNF receptors (p55 and p75) showed a changed responsiveness to LPS [88]. When the more complex CLP was applied to mimic real sepsis, it could be shown that the knockout mice had a decreased survival rate, indicating that TNF is needed for an adequate response to infection [88].

IL-1 and its related proteins form a family of cytokines and it is extremely pleiotropic [89]. It is the key inducer of fever and was termed 'endogenous pyrogen'. In the context of this review, it is important that IL-1 induces signaling via the IL-1 receptor that also utilizes the signal transducer MyD88 mentioned above, leading to the activation of NF- κ B. Release of IL-1 is preceded by cleavage of an inactive precursor form, which is mediated by the IL-1-converting enzyme also termed caspase-1. Both IL-1 and interleukin-1 β converting enzyme (ICE) knockout mice were generated early leading to a complete lack of IL-1 release. For the IL-1 knockout mouse, it was found that it lacked fever and acute-phase response, and performed better in a model of sterile inflammation induced by turpentine [90]. When challenged with LPS or live *Listeria*, these mice failed to exhibit any difference in survival. Mice lacking ICE in addition were resistant to an experimental septic shock induced by bacterial LPS injection [91]. Furthermore, mice were generated lacking the IL-1 receptor. Although this receptor can also recognize other members of the IL-1 family, for example IL-18, the main importance of this model is a lack of pro-inflammatory activity brought about by IL-1. In contrast to the two models mentioned above, the IL-1R knockout mice were more susceptible to *Listeria* infection, while they exhibited a reduced acute-phase induction and lethality after sterile turpentine induction [92]. When induced by *S. aureus*, these mice also showed a worsened outcome, which included both sepsis, and septic arthritis [93]. In conclusion, according to the animal knockout data, the role of the IL-1 family in sepsis, SIRS and CARS apparently is complex: while IL-1 seems to mediate effects contributing to lethality, other members of this family, as shown by the experiments with the IL-1R knockout mice, seem to also play a protective role during infection.

Anti-Inflammatory Cytokine Knockout Animals

As mentioned above, IL-6, an important cytokine during the SIRS/CARS cascade, has both pro- and anti-inflammatory properties. Mice lacking IL-6 cannot mount an acute-phase response and succumb to infections by *Vaccinia* virus and *L. monocytogenes* [94]. In a model of pneumonia utilizing the most important causative bacterium, *S. pneumoniae*, the authors also found an earlier death in IL-6 knockout animals, and a lack of acute-phase response. However, pro-inflammatory cytokines, such as TNF, IL-1 β , and IFN- γ , as well as of the anti-inflammatory cytokine IL-10 were increased [95].

When the key anti-inflammatory cytokine IL-10 is lacking in mice, an almost uncontrolled inflammatory

response is the consequence. When *E. coli* are injected intraperitoneally in normal mice, IL-10 concentrations in peritoneal fluid, blood and lungs increase in order to limit the inflammatory response. Despite a lower bacterial load, IL-10 knockout mice exhibited higher levels of pro-inflammatory cytokines, more severe organ damage and lethality [96]. Another group introduced the more complex CLP model leading to a peritonitis caused by a multitude of commensal bacteria [97]. Here, after CLP surgical interventions were also performed in order to rescue the animals. It was found that from a certain time point on, rescue was impossible, most likely due to an enhanced and uncontrollable inflammatory response. Confirming the previous findings, the authors found that IL-10-deficient mice were more susceptible to CLP sepsis. Also sepsis in these animals was accompanied by 15-fold higher TNF serum levels as compared to control mice [97]. Consistent with these findings, the efficacy of rescue surgery after CLP was lost 10 h earlier in IL-10 knockout mice as compared to wild-type animals. These results suggest that IL-10 controls the onset of irreversible septic shock after CLP.

Disrupting the TGF- β gene in mice leads to a spontaneous hyper-inflammatory state with wasting and death by the age of 3–4 weeks [98]. Pathological examination revealed an excessive inflammatory response primarily in heart and lungs resembling that of an acute infection. More recently it was found that these animals also had excessive levels of pro-inflammatory cytokines and an elevated expression of TLR4 [99]. Interestingly, these mice, although in a fatal state of (sterile) hyper-inflammation, were resistant to experimentally induced endotoxin shock.

Overall, the data concerning animal models of genetic influence in SIRS and CARS lead to the conclusion that genetic disruption of pro-inflammatory pathways seems to blunt the response to predominantly pharmacological stimulation with components of bacteria, whereas the ability to mount an innate response directed to eliminate bacterial infection is disturbed (table 2). The assumption that genetic influence on innate pathways inhibits the ability to adequately fight infections seems to be related to the extent of pro-inflammation, where animals unable to release pro-inflammatory cytokines sufficiently are disadvantaged. This would imply that a reduction in pro-inflammatory activity could be advantageous if the stimulus is non-infectious, but the reduction of activity could equally impair the ability to fight infection.

Fig. 1. Balance of the inflammatory response as determinant of SIRS and CARS, potential influence of genetic variation. Proposed mechanism for the interplay of SIRS and CARS in patients following an insult (**a**). It is proposed that the initial pro-inflammatory reaction (SIRS) induces the compensatory reaction (CARS). However, an independent induction of the anti-inflammatory cascade is also possible. Genetic influence and subsequent modulation of the response is marked by different gray shades in the curves of the diagram (**b**). Extent of the response is depicted through the area under the curve. Identical areas under the curve would indicate a balanced response. Any imbalance could lead to the proposed outcomes.

Discussion

SIRS and CARS are known to depend on each other in many ways. The main debate arises from the pivotal question whether the magnitude of SIRS controls the extent of CARS or if they start independently with the same trigger. Another question is in which manner SIRS and CARS depend on the form of insult, being infections or sterile, such as trauma or extensive elective surgery. Many studies of genetic influence on sepsis focus on levels of pro-inflammatory cytokines. So how tightly is CARS regulated by the pro-inflammatory cytokines? Negative or positive feedback may furthermore influence either one of them. At this point, genetic influence might either aggravate or dampen the response to an inflammatory stimulus.

Genetic variation may influence CARS either directly by affecting sensing or signaling in anti-inflammatory

pathways or indirectly by affecting the primary pro-inflammatory response and thus enhancing or decreasing the secondary anti-inflammatory process. Figure 1 gives an overview of possible scenarios of genetic influence in SIRS and CARS and tries to depict a potential interdependence of the SIRS and CARS response.

Anti-inflammation is not the same as immunosuppression, therefore factors leading to immunosuppression are most likely anti-inflammatory, but this assumption is not necessarily the truth. Two recent studies may help in understanding the process of SIRS, CARS and immunosuppression. The first one showed that mice challenged with intra-abdominal infections by CLP followed by treatment could be distinguished through their cytokine release pattern into 'early' and 'late' death groups [100]. It would be highly interesting to see if knockout animals would show distinct patterns indicating which

factor might be mainly involved in the pathophysiology of immunosuppression following an insult.

In the second study, it was shown that some very distinct patterns of pro- and anti-inflammatory cytokine release happen in patients suffering from community-acquired pneumonia. There was a correlation between the extent of pro-inflammatory TNF and IL-6 levels with anti-inflammatory IL-10 levels that were indicative of the outcome [101]. This may indicate that a correlation between high and low levels in each cytokine category indicates outcome. It would be of great interest to see whether this is associated with genetic factors. However, despite the fact that this study was designed to elucidate genetic factors, these data are lacking to date.

Today one school of thought is that sepsis trials have been hampered by the inclusion of sub-groups differing in pathophysiology and outcome of disease. Dividing the large sepsis cohorts into sub-groups according to their genetic profile could be a first step towards 'individualized medicine' with a potentially more successful therapeutic strategy. Particularly when it becomes clear that discriminating between SIRS and CARS is important for successful therapy, a given patient's SNP profile of certain genes of interest could potentially aid in decision making with regard to timely administration of substances influencing the inflammatory process, especially in critically ill patients [102]. To achieve this goal there are still matters of debate like issues of data safety in genotyping, disease definitions and the design and development of suitable animal models [103, 104]. The question of whether target genes ('candidate gene approach') or genome-wide association studies with the description of single risk factor versus genomic profiles will help the patient's needs best is today still unanswered. Since the systemic re-

sponse consists of a complex biological network, it seems very difficult to achieve such a goal, which then has still to be translated into practical medicine [105].

Further questions that in the future may be answered by employing genetic studies in humans are: Is systemic inflammation or compensatory anti-inflammation an evolutionary 'problem'? Is systemic inflammation beneficial from that perspective and has selective pressure led to advantageous genetic variations present today? What are the advantages of 'excessive' systemic inflammation as opposed to a 'controlled' reaction? Is there an evolutionary advantage to mount a reduced early alarm reaction towards unspecific stimuli that in the event of infection leads to harm by the inability to contain infection? [106].

Conclusion

Genetic variants are very common in mammals and thus variants in the immune system are very likely to contribute to the response of the individual host to any insult. It is clear that the phenomena of SIRS and CARS like almost all events during the immune reaction are determined by genetic factors. The question is whether genetic variations confer a beneficial or detrimental effect with regard to clinical outcome of the patients. To date more and more results are surfacing pointing towards a hereditary determination of common diseases and inflammatory states. However, the problems of studying SIRS and CARS are far from being resolved since commonly accepted definitions of CARS are lacking and animal models are too heterogeneously performed to produce solid conclusions.

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