Pediatric Sepsis: Genetic Considerations

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Abstract

The mortality of childhood sepsis continues to be rather high. When it comes to prevention and adequate therapy, individual differences and genetic alterations are becoming more and more important. These may affect molecules involved in pathogen recognition (e.g., lipopolysaccharide-binding protein, mannose-binding lectin, bactericidal/permeability-increasing protein, Toll-like receptors), signal transduction pathways (e.g., cRel), proinflammatory (e.g., tumor necrosis factor-α, interleukin-1 [IL-1], IL-6, IL-8) as well as anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-1 receptor antagonist), members of the coagulation cascade, and other molecules active in the process of systemic inflammatory response syndrome (e.g., heat shock proteins, complement system). The most common genetic polymorphisms are the so-called single-nucleotide polymorphisms, which entail the change of a single base. Genetic mutations have an impact on susceptibility, severity, and outcome of sepsis. Understanding such mutations may improve treatment efficiency; although there is a considerably limited choice of causal treatments today, they may become available upon future developments in genetic therapy.

Keywords
- sepsis
- infection
- genetics
- childhood
- polymorphism
- single-nucleotide polymorphism
- review
- systemic inflammatory response syndrome

Introduction

In addition to several other factors (e.g., type and virulence of pathogen, general condition of host, immune status), a substantial role is attributed to genetic factors in the development of pediatric sepsis. Several studies have found an association between changes in genes encoding systemic inflammatory response syndrome (SIRS) mediators and sepsis outcome. Some genetic variants can cause structural changes in proteins or changes in the amount of protein produced. Most commonly, this variability only affects a single nucleotide (single-nucleotide polymorphism, SNP). Genetic variants influence the body's susceptibility to the development of sepsis and modify the processes of normal systemic inflammatory response, impairing, or, on the contrary, improving disease outcomes. During SIRS, various causal factors (e.g., trauma, inflammation, infection) trigger the synthesis of proinflammatory, for example, tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-8, and complement factors C3 and C5, as well as anti-inflammatory cytokines, for example, IL-4 and IL-10. Interestingly, genetic changes in components of the coagulation cascade influence the prognosis of sepsis and may affect molecules involved in pathogen recognition, pro- and anti-inflammatory cytokines, and other effector proteins (►**Table 1**).

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Molecules Involved In Pathogen Recognition

Lipopolysaccharide-Binding Protein

Lipopolysaccharide-binding protein (LBP) plays an important role in pathogen recognition and hence in triggering the immune response. First binding to gram-negative bacterial lipopolysaccharide (LPS) to form a complex, LBP is then bound to Toll-like receptor (TLR)-4/CD14/MD2 receptors of macrophages, thereby activating these immunocytes.[1,2] A total of 112 SNPs have been identified in the *LBP* gene, the most important being rs2232618 (T1412C, Phe436Leu). A study conducted in a Chinese population has found that this SNP increases sepsis and multiple organ failure (MOF) incidence rates in relatively severe posttraumatic patients.[3] Although the study involved an adult population, it is presumable that similar findings would be obtained in pediatric subjects.

Bactericidal/Permeability-Increasing Protein

Bactericidal/permeability-increasing protein (BPI) is primarily found in azurophilic granules of neutrophil granulocytes, but also detectable on the cellular surface and in specific granules of eosinophil granulocytes.[4] It has a role in the defense against gram-negative bacteria by damaging external and internal bacterial membranes and neutralizing LPS, and, on the other hand, playing a part in opsonization.[5–7] Two frequent polymorphisms have been identified, including the mutation known as BPI Taq (G545C), a so-called silent mutation since causing no change to the encoded amino acid (Val182).[8] It has, however, been found to be associated with sepsis outcome in children, leading to a hypothesis that it may be a marker of other, hitherto unknown functional variants with which it is in a strong linkage disequilibrium. The homozygous GG allele was present in 85% of children with septic shock. No such association is currently known for the other SNP, BPI 216 (A645G, Lys216Glu).[9]

NOD2/CARD15

The NOD2 molecule recognizes bacterial particles (e.g., peptidoglycans) and stimulates immune response.[10] Its most important polymorphism is the Leu1007fsinsC mutation, which causes the produced protein to be unable to activate the nuclear factor κB (NF-κB) molecule.[11] This leads to a slowdown of monocytic phagocytosis, which contributes to the development of bacteremia and sepsis.

Toll-like Receptors

TLRs constitute a part of the innate immune system. To date, as many as 11 types have been described.[12] They recognize what is referred to as "pathogen-associated molecular patterns."[13] TLR signal pathways elicit the activation of common transcription factors (NF-κB and mitogen-activated protein kinases [MAPKs]) and result in the production of cytokines IL-6, IL-10, and IL-12, as well as the costimulatory molecules CD40 and B7. However, although various TLRs bring similar or identical molecules (MyD88, NF-κB) into action during their activation, they can elicit immune responses that are different and unique to a single TLR type.

Toll-like Receptor 1

TLR-1 is found in membranes of macrophages, dendritic cells, eosinophils, basophils, and mast cells, forming a heterodimer with TLR-2. Of the two most common SNPs of the *TLR-1* gene, $TLR1_{-7202A/G\ (Asn248Ser)}$, which is found in the 5′ nontranscribed region of the *TLR* gene, shows a stronger association with clinical outcome than $TLR1_{1804G/T\ (Ser602Ile)}$, which encodes a protein located in the transmembrane domain.[14] $TLR1_{-7202G}$ is a hypermorphic allele strongly associated with sepsis-related organ damage and mortality in the American population.[15] Increased receptor density on the cellular surface owing to the mutation is thought to be responsible for this effect. In the presence of the $TLR1_{1804T\ (602Ile)}$ allele, NF-κB induction is intensified in *Staphylococcus aureus* infection.[16] With another SNP, $TLR1_{1805G/T}$, when the T allele was present in a homozygous form, intensive care unit length of stay significantly increased in septic children whose blood cultures were positive. Compared with G/G homozygous patients, length of stay was also longer in G/T heterozygous cases, which is explained by an increase in neutrophil activation rates when the T allele is present.[17]

Toll-like Receptor 2

TLR-2 is the most important member of the receptor family, having a role in the recognition of various bacterial lipoproteins.[18] Several studies have proven that TLR-2 has a fundamental role in antibacterial defense. The TLR-2 gene's best known polymorphism is TLR-2 Arg753Gln, which inhibits tyrosine phosphorylation, dimer formation with TLR6, and the buildup of MyD88, and affects NF-κB activation.[19,20] Consequently, peptidoglycan- and lipopeptide-triggered intracellular signal transduction and cytokine secretion are compromised, leading to a weakened immune response, which may result in the onset of severe sepsis.[21] Although a study on a smaller group of Asian adults detected no clear association, and no major pediatric studies were completed so far, it is presumed that findings similar to those in adults would be obtained in children. The role in sepsis of another polymorphism, TLR2 Arg677Trp, has not been investigated, but it is clear that this predisposes children to infections caused by *S. aureus* and other pathogens.

Toll-like Receptor 4

As part of the innate immune system, TLR-4 plays an important role in the defense against gram-negative bacteria, but it also recognizes mycobacterial and fungal proteins, as well as being activated by endogenous ligands. Compounds synthesized due to the signal transduction process triggered by TLR-4 include TNF-α, IL-1β, and interferon-β (IFN-β), among others. There are two polymorphisms known to affect receptor function, Asp299Gly (rs4986790) and Thr399Ile (rs4986791), which cause cytokine synthesis rates to drop, with a consequential increase in susceptibility to gram-negative bacteria.[22] Results concerning a link between these SNPs and sepsis are controversial; haplotype Asp299Gly/Asp299Gly seems to induce enhanced TNF-α synthesis in response to LPS, thus predisposing to sepsis development, whereas haplotype Asp299Gly/Thr399Ile has no or limited

Table 1 The most important genetic polymorphisms in sepsis

Gene	Polymorphism	Consequence	References
LBP	rs2232618 (T1412C, Phe436Ile)	Increases sepsis and MOF incidence rates in relatively severe posttraumatic patient	3
BPI	Taq (G545C)	The GG allele associates with sepsis outcome in children	9
NOD2/CARD15	Leu1007fsinsC	The produced protein unable to activate the NF-κB	11
TLR-1	−7202A/G (Asn248Ser)	Strongly associated with sepsis-related organ damage and mortality	15
	1805G/T	The TT allele increases the intensive care unit length of stay in septic children	17
TLR-2	Arg753Gln	Inhibits tyrosine phosphorylation, dimer formation with TLR-6, and the buildup of MyD88, and affects NF-κB activation	19,20
	Arg677Trp	Predisposes children to infections caused by *Staphylococcus aureus*	
TLR-4	rs4986790 (Asp299Gly); rs4986791 (Thr399Ile)	Cause cytokine synthesis rates to drop, with a consequential increase in susceptibility to gram-negative bacteria	22
	haplotype Asp299Gly/ Asp299Gly	Induces enhanced TNF-α synthesis in response to LPS, predisposes to sepsis development	23–25
	rs1927907	Plays a role in the development of *S. aureus* sepsis	26,27
CD14	rs2569190 (−159C/T)	Leads to increased CD14 levels	32
MBL	B,C,D variants	Causes the protein's serum levels to drop	35–38
cRel	rs842647 (A61119471G), rs13031237 (G61136129T)	rs842647*G increases the risk of MOF in septic patients	40
TNF-α	−308G/A	TNF2 (A) allele increases TNF-α secretion and mainly affects sepsis onset risks rather than outcomes	43–45
TNF-β	TNFB1, TNFB2	TNFB1 causes elevated serum levels, and hence increased mortality, in septic children	47
IL-1α	−889C/T	Causes elevated IL-1α expression, which some studies describe as being associated with sepsis	52
IL-1β	+3954C/T	Changes splice donor site, leading to the synthesis of fragile, inactive molecules; the T allele increases IL-1β production induced by LPS; the presence of TT alleles carries a reduced risk of sepsis	52–54
	−511G/A	Elevates IL-1β levels in response to endotoxins	
	−31C/T	Affects transcription factor binding sites, hence transcription activity	
IL-6	−174G/C	The C allele is associated with lower plasma levels; the G allele seems to be protective against sepsis	62,63
	Haplotype variants (−174/1753/2954)	Increase mortality or MOF	64
IL-8	−251T/A (rs4073)	Significantly increases the risk of severe sepsis in newborns; the T allele is protective against sepsis in women	66
	rs1126647	The A allele is protective against sepsis in women	70
	rs2227306	The C allele represents an elevated risk of sepsis in men	
IFN-γ	−1616T/C (rs2069705)	The T allele has a protective effect against sepsis development	72
	+3234C/T (rs2069718)	The C allele protects from severe sepsis	
	−1616/−764/ + 874 haplotype	The CTT haplotype is protective against sepsis; TAC haplotype is associated with susceptibility to sepsis and protects against severe sepsis	
HMGB1	1377delA	Significantly makes worsen late-phase sepsis mortality	76
	982C/T	Increases early mortality risk in sepsis	
	rs1045411 (2262G/A)	The A allele causes far more severe inflammatory response than the GG genotype	77

Table 1 (*Continued*)

Gene	Polymorphism	Consequence	References
IL-1RA	86 base-pair-long VNTR	The A2 allele causes elevated IL-1α, and even higher IL-1β levels; the resulting lower IL-1α:IL-1β ratio leads to more intensified and lengthier inflammatory response and also results in reduced IL-1RA levels in severely septic patients	79–81
IL-10	−1082(A/G)	The A allele is associated with susceptibility to sepsis, whereas the G allele causes enhanced IL-10 production and increases mortality in severe sepsis	83,84
PC	(−1654C/T, −1641A/G) single haplotype	The homozygous GC genotype is associated with significantly lower PC levels increasing the propensity to thrombosis in sepsis; it also worsens outcomes by causing hypotension The homozygous TA genotype has a protective effect on sepsis outcomes The genotype −1641A/A, worsens survival and aggravates systemic inflammation	89–91
PAI-1	−675 4G/5G insertion/deletion	The 4G/4G genotype results in elevated plasma levels and activity and increases susceptibility to sepsis and mortality in children with meningococcal infection, as well as raises the incidence of DIC	93,94
Fibrinogen	−854G/A, −455G/A, +9006G/A	The GAA haplotype causes elevated fibrinogen levels owing to the −455A allele, yet it reduces mortality and MOF incidence in sepsis	100–103
FcyRIIa	R131H	The R131 allotype causes the receptor to bind the IgG2 molecule with a reduced affinity, which results in a slowdown of IgG2-opsonized phagocytosis	110–112
FcyRIIIa	Val158Phe	Modifies the receptor's affinity to IgG1, IgG2, and IgG4	116,117
FcyRIIIb	switch in four amino acids (Na1/Na2),	Affects the extracellular domain and has a role in protein glycosylation and hence the phagocytosis of particles opsonized by IgG1 and IgG3	118–120
CFH	Y402H	Has a protective effect because inhibition of the alternative pathway becomes muted and has a higher baseline level of activity	131
	−496C/T	The CC genotype, accompanied by elevated CFH levels, is a factor of increased risk for meningococcal infection	132
MIF	−173 G/C (rs755622), −794 $CATT_{5-8}$ (rs5844572)	CATT7/8 is associated with severely complicated malaria, mortality of severely septic patients, and survival in community-acquired pneumonia The low-expression allele (CATT5) has a protective effect against meningococcal infection in children Haplotype −173C/−794CATT7 may help identify patients at increased risk of sepsis mortality	137–139,143
HSPA1A	rs1008438	The A allele causes hematological impairment	147
	rs1043618	The C allele leads to longer intensive care unit treatment periods in H1N1 infection and to a higher incidence of liver failure and MOF in severe trauma cases	
HSPA1B	1538G/A	The A allele causes elevated TNF-α and IL-6 levels	146
	1267A > G (rs1061581)	Increases the risk of sepsis in adults with community-acquired pneumonia The AA genotype is clearly associated with sepsis secondary to community-acquired pneumonia The A allele causes hematological impairment	147,148
HSPA1L	2437C > T (rs2227956)	The C allele causes elevated TNF-α and IL-6 levels and is also a significant risk factor for liver failure and MOF	146
HSP70–2	HSP70–2A/G	The G allele is associated with lower protein levels and represents an increased risk for sepsis The A allele, a link has been found with TNF-β2, which worsens outcomes in sepsis	149
DDAH2	−449G/C	The −449G allele has been shown to result in lower ADMA levels and is more likely to be present in sepsis accompanied by "cold shock"	156

Abbreviations: ADMA, asymmetric dimethylarginine; BPI, bactericidal/permeability-increasing protein; CFH, complement factor H; DDAH, dimethylarginine dimethylaminohydrolase; DIC, disseminated intravascular coagulation; HMGB1, high mobility group box 1; IFN-γ, interferon gamma; IgG, immunoglobulin G; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; MBL, mannose-binding lectin; MIF, macrophage migration inhibitory factor; MOF, multiple organ failure; NF-κB, nuclear factor κB; PAI-1, plasminogen activator inhibitor 1; PC, protein C; TLR, Toll-like receptor; TNF, tumor necrosis factor; VNTR, variable number of tandem repeats.

effect on receptor function.[23–25] Selected studies conclude that rs1927907 polymorphism plays a role in the development of *S. aureus* sepsis; in this case, the receptor is activated by substances other than LPS, indicating that TLR-4 probably also responds to endogenous ligands.[26,27]

CD14

The CD14 protein has an important role in the recognition of gram-negative bacteria since it activates the NF-κB pathway by binding to TLR4 as part of the LPS–CD14–MD2 complex.[1] CD14 is found on the surface of monocytes, macrophages, and neutrophil granulocytes in a membrane-linked form, but a soluble form also exists.[28] Elevated CD14 levels pose an increased risk to sepsis and MOF.[29,30] Many polymorphisms of the gene are known; the most commonly described mutation being rs2569190 (-159 C/T) in the promoter region, leading to increased CD14levels.[31] Its association with sepsis is controversial; although several studies have reported about a link between this polymorphism and sepsis, mainly in the Asian population, but further studies are needed for a clear standpoint in this issue.[32]

Mannose-Binding Lectin

Mannose-binding lectin (MBL) is a selectin molecule, which, by binding to mannose and bacterial N-acetyl galactosamine components, activates the complement system through the lectin pathway.[33] There are three known polymorphisms of the *MBL-2* gene at the codon 52, 54, and 57 (referred to as variants B, C, and D, respectively, or collectively as O, whereas the wild type denoted as A). The mutations cause an amino acid switch, which interferes with oligomerization, causing the protein's serum levels to drop. Patients with MBL deficiency are more susceptible to sepsis. MBL levels are lower in A/O heterozygosis, whereas circulatory MBL is almost completely absent in O/O homozygous or compound heterozygous cases.[34] Added to these three mutations, there are three major polymorphisms in the promoter region, one of which (-221G/C, also known as Y/X) is a major factor of MBL expression.[35] The three polymorphisms in exon 1 are in linkage disequilibrium with the promoter X/Y polymorphism, with Y being the only variant with a link to each.[36,37]

Polymorphisms Affecting Signal Transduction Pathways

cRel Polymorphism

The cRel protein is a subunit of NF-κB. The latter is a protein complex with a central role in the body's response to infections. It is activated through two possible pathways. The canonical pathway involves TLR as well as proinflammatory cytokines (e.g., TNF-α, IL-1) activating the RelA protein, which has proinflammatory effects and activates genes responsible for the cell's survival.[38] The other one is an alternative pathway initiated by LTb, CD40L, BAFF, and RANKL, followed by activation of the RelB/p52 complex, resulting in transcription of genes responsible for lymph organogenesis and B-cell activation. There are two important polymorphisms of the cRel gene: rs842647 and rs13031237; the former one leading to an A→G substitution at location 61119471 in intron 2, whereas the latter eliciting a G→T switch at location 61136129, intron 4, chromosome 2. It has been shown that the minor allele rs842647*G increases the risk of MOF in septic patients, whereas no association with the severity of septic shock has been found for the allele rs13031237*T.[39]

Proinflammatory Cytokines

Tumor Necrosis Factor-α, Tumor Necrosis Factor Receptor

TNF-α is a proinflammatory cytokine involved in the process of SIRS and in initiation of the acute phase reaction. Dominantly produced by activated macrophages, TNF-α can also be a product of CD4+ T-lymphocytes, neutrophils, mast cells, eosinophilic granulocytes, and neurons. Its primary function is immune cell regulation. Bound by either of two receptors, TNFR1 and TNFR2, TNF-α can then activate three pathways: NF-κB, MAPK, and the so-called cell-death pathway. Many SNPs at the TNF/LTA locus have been identified as affecting TNF-α production and being associated with sepsis onset or outcomes.[40] The most commonly known mutation is -308G/A; notable examples also include the polymorphisms -238G/A and LTA (TNF-β) +249 and +365.[41] The TNF-α -308G/A polymorphism affects the promoter region and causes one TNF1 (G) and one TNF2 (A) allele to be present, the latter leading to increased TNF-α secretion, a condition mainly affecting sepsis onset risks rather than outcomes.[42–44] As for -238G/A SNP, the -238A allele results in reduced TNF-α levels.[45] The associated TNF-β polymorphism entails a TNFB1 and a TNFB2 allele, with the latter causing elevated serum levels, and hence increased mortality, in septic children.[46]

Interleukin-1

Members of the IL-1 family have an important role in inflammatory responses and the pathomechanism of sepsis. The family includes one anti-inflammatory and two proinflammatory (IL-1α and IL-1β) cytokines.[47–49] The genes are in a cluster on the long arm of chromosome 2 (2q13–21).[50] A total of five SNPs that can be linked to sepsis are known in the *IL-1* gene. These include -889C/T in the promoter region of the *IL-1α* gene, -511G/A and -31C/T in the promoter region of the *IL-1β* gene, and +3954C/T in exon 5.[51] +3954C/T polymorphism causes no amino acid switch, but changes splice donor site, leading to the synthesis of fragile, inactive molecules.[52] The T allele increases IL-1β production induced by LPS.[53] It has also been shown that the presence of TT alleles carries a reduced risk of sepsis; however, this is inconsistent with the observation that elevated IL-1β levels represent greater susceptibility to sepsis. Additional factors are presumed to be in the background of this controversy; further studies into the link between this polymorphism and sepsis are necessary. The IL-1α -889C/T mutation causes elevated IL-1α expression, which some studies describe as being associated with sepsis. Although IL-1β -511G/A elevates IL-1β levels in response to endotoxins, and IL-1β -31C/T affects

transcription factor binding sites and hence transcription activity, the link between these SNPs and sepsis remains controversial.[51]

Interleukin-6

IL-6 is a proinflammatory cytokine playing an important role in evolvement of the inflammatory response. *IL-6* gene polymorphisms may have a role in the development and outcomes of childhood sepsis.[54,55] It has been reported that in the IL-6 -174G/C mutation, the C allele is associated with lower plasma levels; however, the results are unclear, with some researchers having found such relationship in newborns only, and other studies pointing out that plasma levels of IL-6 variants depend on sex, age, body mass index, and general health.[56–59] An association with better survival has been detected in GG homozygous cases, and the G allele seems to be protective against sepsis.[60,61] Moreover, reports also describe a link between haplotype variants (e.g., -174/1753/2954) and increased mortality or MOF.[62] The other polymorphism is -572G/C, where the C allele causes a similar deviation in plasma levels compared with the earlier polymorphism.[59] However, the relationship of this variant with sepsis remains unknown.

Interleukin-8

IL-8, also known as neutrophil chemotactic factor, is a chemokine produced by macrophages, epithelial cells, airway smooth muscle cells, and endothelial cells. It has two primary functions including chemotaxis induction in neutrophil and other granulocytes, and having a role in phagocytosis induction and angiogenesis. Elevated IL-8 levels are associated with the development of severe sepsis.[63] Baseline IL-8 level is one of the most important prognostic factors for sepsis outcome.[64] There is a link between *IL-8* gene polymorphisms and sepsis onset; SNP IL-8 −251T/A (rs4073) has been described to significantly increase the risk of severe sepsis in newborns.[65] The findings are controversial, with some studies identifying the T allele as causing elevated IL-8 levels and others claiming the same about the A allele even examples of a failure to detect a difference in IL-8 levels for the different alleles that exist.[66–68] However, the T allele has been found to be protective against sepsis in women. The same was identified in rs1126647 mutation for the A allele. In both cases, a greater incidence of the protective allele was confirmed in women. As for the rs2227306 mutation, the C allele represented an elevated risk of sepsis in men.[69]

Interferon Gamma

INF gamma (IFN-γ) is a cytokine that is an important component of the innate and acquired immunity with a primary role in antiviral defense and an activity against selected bacterial and protozoal species. It is mainly produced by NK and NKT cells, but can also be synthesized by CD4+ Th1 and CD8+ cytotoxic T cells. IFN-γ is an important activator of macrophages and induces major histocompatibility complex II (MHCII) expression. Its importance is rooted in its direct viral replication inhibitory capacity, as well as immunostimulant and immunomodulatory effects.[70]

Highlights of *IFN-γ* gene polymorphisms include four entities with links to immunological pathologies. Two of these, -1616T/C (rs2069705) and -764G/C (rs2069707), are found in the promoter region, with another located in intron 1 (+874A/T [rs2430561]) and yet another in intron 3 (+3234C/T [rs2069718]). Alleles -1616T, -764G, +874A, and +3234C have been shown to cause elevated IFN-γ levels compared with their counterpart variants. Reports also indicate that the SNPs +874 and +3234 are in a "linkage disequilibrium" with each other. Studies have demonstrated that the -1616T allele has a protective effect against sepsis development and that the +3234C allele protects from severe sepsis. Research into the haplotypes of -1616, -764, and +874 found that the CTT haplotype is protective against sepsis, but only minimally protective against the development of severe sepsis, whereas the TAC haplotype is associated with susceptibility to sepsis and protects against severe sepsis. The TAC haplotype comprises three alleles encoding enhanced gene expression and represents the most common combination, with CTT being the rarest. Patients whose IFN-γ expression is enhanced have a greater propensity to develop sepsis but are at a lower risk of severe sepsis, whereas those with lower IFN-γ expression rates are less prone to sepsis.[71]

High Mobility Group Box 1

High mobility group box 1 (HMGB1), also known as nuclear nonhistone DNA-binding protein, is a multifunctional cytokine with a role in late-phase inflammatory response. It is mainly produced by macrophages and monocytes in response to LPS through a TNF-α-dependent mechanism, but it is also released by necrotic but not apoptotic cells, which is a way for the body to differentiate between these cells.[72,73] In addition to its cell-damaging effect, HMGB1 also has a protective function in that it elicits stem cell migration into inflamed areas, promoting regeneration.[74] HMGB1 levels are significantly higher in septic patients, and in mice model, anti-HMGB1 antibodies significantly reduce mortality.[73] The *HMGB1* gene has many polymorphisms, two of which have a significance in sepsis; one such mutation is -1377delA, which has been shown to significantly worsen late-phase sepsis mortality. The other polymorphism is 982C/T, which is found in exon 4 and is associated with significantly lower HMGB1 levels than the homozygous 982C/C genotype, thereby increasing early mortality risk in sepsis.[75] Research in Korean children has found that the A allele in rs1045411 (2262G/A) polymorphism causes far more severe inflammatory response than the GG genotype.[76]

Anti-Inflammatory Cytokines

Interleukin-1 Receptor Antagonist

As suggested by its name, the IL-1 receptor antagonist binds to the IL-1 receptor to inhibit its function. There is an 86-base-pair long VNTR (variable number of tandem repeats) sequence in intron 2 of the IL-1 receptor antagonist (IL-1RA) gene, involving five different alleles (1–5) of different repeat frequencies (4, 2, 5, 3, and 6 repetitions). These are further

classified into long (L: 1, 3, 4, 5) and short (2 only) genotypes, with possible combinations including L/L, L/2, and 2/2.[77] The A2 allele causes elevated IL-1α, and even higher IL-1β levels. The resulting lower IL-1α:IL-1β ratio leads to more intensified and lengthier inflammatory response.[78,79] This allele also results in reduced IL-1RA levels in severely septic patients.[80] All this indicates that this polymorphism plays an important role in immune response regulation.

Interleukin-10

L-10, also known as "human cytokine synthesis inhibitory factor," is an anti-inflammatory cytokine with a central role in immunoregulation and inflammatory processes. Its functions include down-regulation of cytokines expressed by Th1 cells, moreover that of MHCII antigens and macrophageal costimulant molecules.[81] It also promotes B-cell survival, proliferation, and antibody production. IL-10 inhibits NF-κB activity and has a role in regulation of the JAK-STAT signal pathway. Several IL-10 polymorphisms are known, of which SNP -1082(A/G) in the promoter region has been linked to sepsis. The presence of the A allele is associated with susceptibility to sepsis, whereas that of the G allele causes enhanced IL-10 production and increases mortality in severe sepsis.[82,83] No such relationship has been found for the other two frequent polymorphisms (-592 and -819).[84]

Polymorphisms Affecting the Coagulation System

The coagulation system is activated in sepsis in response to bacterial endotoxins, resulting in enhanced expression of tissue factor in monocytes and endothelial cells, thus activating the extrinsic pathway of the coagulation cascade. However, sepsis essentially leads to complex dysregulation of procoagulant, anticoagulant, and fibrinolytic proteins. The presence of various gene polymorphisms is also important in relation to the outcome of sepsis-related disseminated intravascular coagulation (DIC).

Protein C

Protein C (PC) is a vitamin K dependent zymogenic serine protease synthesized in the liver; once activated and bound to thrombomodulin, it inactivates factors Va and VIIIa in the presence of protein S, PL, and Ca^{2+}, thereby blocking any further production of thrombin. It also has anti-inflammatory and antiapoptotic effects.[85] Of the polymorphisms of the *PC* gene, two located in the gene's 5′ nontranscribed region (-1654C > T, -1641A > G) bear importance.[86,87] These two polymorphisms form a single haplotype where the homozygous GC genotype is associated with significantly lower PC levels, increasing the propensity to thrombosis in sepsis; it also worsens outcomes by causing hypotension.[88] The homozygous TA genotype has a protective effect on sepsis outcomes because the higher PC levels decrease the incidence of DIC in sepsis, thereby improving survival.[89] The genotype -1641A/A, however, worsens survival and aggravates systemic inflammation.[90]

Plasminogen Activator Inhibitor 1

The plasminogen activator inhibitor 1 (PAI-1) molecule is a serine protease inhibitor blocking tissue plasminogen activator (tPA) and urokinase plasminogen activator, the activators of plasminogen and hence fibrinolysis; it also forms a complex with activated PC (APC) to inhibit the latter's function. Based on this behavior, PAI-1 has a procoagulant effect. Several polymorphisms of the *PAI-1* gene are known. The most frequently studied polymorphism with an outstanding importance regarding sepsis is the -675 4G/5G insertion/deletion polymorphism in the promoter region, affecting PAI-1 plasma levels and activity.[91] It has been shown that presence of the 4G/4G genotype results in elevated plasma levels and activity and increases susceptibility to sepsis and mortality in children with meningococcal infection, and also raises the incidence of DIC.[92,93] Its further consequences include elevated risks of septic shock and MOF in pneumonia-induced sepsis.[94] The significance of this genetic variant lies in the fact that the administration of tPA and APC is a therapeutic option that might improve outcomes.[95–98]

Fibrinogen

Fibrinogen, or factor I, is synthesized in the liver. As part of the coagulation cascade, it is converted into fibrin by thrombin. Of the polymorphisms of the *fibrinogen-β* gene, research into -854G/A, -455G/A, and +9006G/A revealed that even though the GAA haplotype causes elevated fibrinogen levels owing to the -455A allele, it reduces mortality and MOF incidence in sepsis.[99–102] This might be explained by the fact that in addition to its role in coagulation, fibrinogen also takes part in the inflammatory process since it is a member of the acute phase proteins; it promotes neutrophil and IL-8 secretion as well as exerts an antiapoptotic effect on endothelial cells and neutrophils.[103–107]

Effector Molecules

Fcy Receptors

Fcy receptors earned their name by binding the Fc region of immunoglobulin G (IgG). They can also be found on many immune and nonimmune cell types. Their task is to stimulate phagocytes or cytotoxic cells, which destroy pathogens or infected host cells by antibody-mediated phagocytosis or through a mechanism referred to as antibody-dependent cell-mediated cytotoxicity. They have three subclasses, FcyRI (FcyrIa), FcyRII (a, b, c), and FcyRIII (a, b), each with a different level of affinity to various IgG antibodies. Several polymorphisms affecting receptor function have been described in these three groups.[108] The most important polymorphism of the *FcyRIIa* gene results in an arginine–histidine switch at position 131 of the protein (R131H); the R131 allotype causes the receptor to bind the IgG2 molecule with a reduced affinity, which results in a slowdown of IgG2-opsonized phagocytosis.[109–111] IgG2 is the major antibody subtype in the defense against encapsulated bacteria (e.g., *Neisseria meningitidis*); this mutation increases the susceptibility for these pathogen.[109,112,113] The most significant polymorphism of FcyRIIIa causes a valine–phenylalanine switch at position 158, which

modifies the receptor's affinity to IgG1, IgG2, and IgG4.[114,115] For FcyRIIIb, the polymorphism causes a switch in four amino acids (Na1/Na2), which affects the extracellular domain and has a role in protein glycosylation and hence the phagocytosis of particles opsonized by IgG1 and IgG3.[116–118] Phagocytosis is more efficient in FcyRIIIb Na1/Na1 homozygosity. Research into the polymorphisms FcyRII H131 and FcyRIIIa Na2 has revealed that both are associated with an increased susceptibility to meningococcal infection.[119–124] Although the results are controversial, most studies confirm these findings.

Other Molecules

Complement System

The complement system is part of the innate immune system that enhances (complements) the functions of antibodies and phagocytic cells, thus helping to eliminate pathogens and remove damaged cells; on the other hand, it promotes inflammation and directly damages pathogen's membranes.[125] Its activation may occur through three pathways: the classical, lectin, and the alternative pathways.[126] It is important to note that the alternative pathway is constantly activated for antibacterial defense and that it also enhances the other two pathways.[127] The regulatory role of complement factor H (CFH) is extremely important in preventing uncontrolled complement activation and excess damage. CFH levels gradually rise from as early on as the acute phase of inflammation.[128] The effect of complement factor B is the exact opposite, that is, potentiating function of the alternative pathway. The classical and lectin pathways are activated by the C1q protein and MBL, respectively. Gene polymorphisms in components of the complement system affect normal functioning of the system. Such mutations most commonly affect CFH. By inhibiting CFH function, CFH Y402H polymorphism has a protective effect because inhibition of the alternative pathway becomes muted and has a higher baseline level of activity.[129] The CFH -496C/T CC genotype, accompanied by elevated CFH levels, is a factor of increased risk for meningococcal infection.[130] MBL-2 polymorphisms increase the risk of sepsis. In summary, mutations that facilitate rapid and efficient activation of the complement system are protective against sepsis.

Macrophage Migration Inhibitory Factor

"Macrophage migration inhibitory factor" (MIF) is an important regulator of the innate immune system.[131] It plays an important role in acute and chronic inflammatory processes and autoimmune diseases. In response to stimulatory effects of bacterial antigens, white blood cells produce MIF,[132] which binds to CD74 molecules on the surfaces of other immunocytes, triggering the acute immune response. MIF is constantly expressed by macrophages and monocytes and has autocrine, paracrine, and endocrine effects when secreted into the extracellular space. Interestingly, glucocorticoids also induce MIF secretion, which, in turn, counters their anti-inflammatory effect. For note, the frontal hypophysis also produces MIF in response to trauma.[133] There are four known polymorphisms to the *MIF* gene. The SNPs +254 (+254*T/C) and +656 (+656*C/G) are in the introns but bear no functional significance.[134] This is in contrast with the other two polymorphisms situated in the promoter region. One of them is an SNP (−173 G/C; rs755622), whereas the other is a microsatellite (−794 $CATT_{5-8}$; rs5844572). The most frequent allele in the Caucasian population is CATT6. Genotypes are classified into a so-called low-expression group with lower MIF levels (5-CATT allele or -173GG) and a high-expression group (7-CATT or the -173C alleles). The -173G allele is much more prevalent than the C allele. Infectious disease studies have shown that high-expression MIF alleles (CATT7/8) or haplotypes are associated with severely complicated malaria, mortality of severely septic patients, and survival in community-acquired pneumonia.[135,136] Also, the low-expression allele (CATT5) has a protective effect against meningococcal infection in children.[137] This suggests that the effects of MIF polymorphisms are modified by age, infection site, pathogen type, and other confounding factors (e.g., selection bias).[138–140] Another study has reported that although the haplotype -173C /-794CATT7 is not useful as a susceptibility marker for sepsis, it may still help identify patients at increased risk of sepsis mortality.[141] The practical significance of research into MIF polymorphisms lies in an ongoing phase 1/2a study for the efficacy assessment of an anti-MIF monoclonal antibody (BAX/imalumab) in patients with metastatic colorectal carcinoma or ovarian cancer with malignant ascites.[134] Potential future use of the antibody might even include the treatment of severe sepsis.

Heat Shock Proteins

Heat shock proteins are members of a family of proteins produced in response to cells being exposed to stressful conditions. Some members of this family have "chaperone" function; they stabilize newly synthesized proteins, facilitate correct folding, and help damaged proteins to refold.[142] As for the immune system, the most important heat shock protein is HSP70 located in the cell membrane because it plays a role in antigen binding and presentation to immunocytes. The gene encoding HSP70 is found in the HLA locus on the short arm of chromosome 6. There are many known polymorphisms to the *HSP70* gene, of which HSPA1A, HSPA1B, and HSPA1L have significance in relation to sepsis. Polymorphisms of the *HSPA1B* and *HSPA1L* genes modify the levels of selected cytokines and thus the process of SIRS. For example, the A allele in HSPA1B 1538G/A, as well as the C allele in HSPA1L 2437 T > C, causes elevated TNF-α and IL-6 levels, and the C allele is also a significant risk factor for liver failure and MOF.[143] It has been reported that 1267A > G mutation in the *HSPA1B* gene increases the risk of sepsis in adults with community-acquired pneumonia.[144,145] The A allele of rs1061581 and the rs1008438 mutation both cause hematological impairment, whereas the C allele of rs1043618 leads to longer intensive care unit treatment periods in H1N1 infection and to a higher incidence of liver failure and MOF in severe trauma cases.[144] Another study points out that AA (rs1061581) genotype of the *HSPA1B* gene is clearly associated with sepsis secondary to community-acquired pneumonia.[145]

This polymorphism is in a strong "linkage disequilibrium" with that of the promoter regions of HSPA1A and HSPA1B.[146] Investigations into the HSP70–2A/G polymorphism found that the G allele is associated with lower protein levels and represents an increased risk for sepsis. For the A allele, a link has been found with TNF-b2, which worsens outcomes in sepsis.[146]

Dimethylarginine Dimethylaminohydrolase

Dimethylarginine dimethylaminohydrolase (DDAH) metabolizes the asymmetric dimethylarginine (ADMA) molecule, which is an inhibitor of inducible nitric oxide synthase.[147] The effects of NO include vasodilation, intense inflammatory response, and reduced thrombocyte and leukocyte adhesion, and it also has a free radical scavenger function. ADMA is synthesized from L-arginine by protein arginine methyltransferase. There is an association between ADMA levels and sepsis.[148] The *DDAH2* gene has two relatively known polymorphisms: -871 6 g/7 g insertion/deletion and the SNP -449G/C.[148–151] The latter has a significance in sepsis since the -449G allele has been shown to result in lower ADMA levels and is more likely to be present in sepsis accompanied by "cold shock."[152]

Discussion

Changes to genes of sepsis mediators have an important role in the susceptibility to the severity and outcome of sepsis. Currently available choices in gene therapy are limited; nevertheless, options do exist and include antibody treatment against abnormally high levels of mediators produced by a mutation (e.g., monoclonal antibodies used against MIF), or potential administration of tPA or APC in genetic alterations of PAI-1. With the advance of molecular genetic methods, genetic testing, even screening, of patient groups at high risk of sepsis, for example, children with chronic diseases (leukemia etc.), may become reality in the future. This would offer a way to reduce the risk of sepsis onset by gene therapy in selected patients carrying mutations; in the interim until such therapy is available, stricter observation and more aggressive sepsis therapy could improve outcomes in these patients. This, however, necessitates further research to clarify in detail the sepsis-related roles of each polymorphism.

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