REVIEW

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Molecular biology on the ICU

From understanding to treating sepsis

J. WINNING [1], R. A. CLAUS [1], K. HUSE [2], M. BAUER [1]

[1]Department for Anesthesiology and Intensive Care Medicine Friedrich-Schiller-University, Jena, Germany
[2]Institute for Molecular Biotechnology (IMB) Genome Analysis, Jena, Germany

Mounting evidence suggests that beside well established factors, such as virulence of pathogens or site of infection, individual differences in disease manifestation are a result of the genetic predisposition of the patient on an Intensive Care Unit (ICU). Specific genetic factors might not only predict the risk to acquire severe infections but also to develop organ dysfunction or ultimately to die. Thus, the advent of molecular techniques allowing screening for a wide variety of genetic factors, such as single nucleotide polymorphisms in genes controlling expression of important mediator systems in patients as well as their purposeful targeting in animal models of sepsis, are revolutionizing understanding of pathophysiology in the critically ill. Molecular tools are about to challenge "state-of-the-art" diagnostic tests such as blood culture as they not only increase sensitivity but dramatically reduce time requirements to identify pathogens and their resistance patterns. Similarly, knowledge of genetic factors might in the near future help to identify "patients at risk", *i.e.* those with a high likelihood to develop organ dysfunction or to guide therapeutic interventions in particular regarding resource-consuming and/or expensive therapies ("theragnostics"). While therapeutic options in molecular intensive care medicine, such as stem cells in the treatment of organ failure or therapeutic gene transfer are possible along the road and might become an option in the future, recombinant DNA technology has already a well defined role in the production of recombinant human proteins from insulin to activated protein C.

Key words: **Molecular biology - Intensive Care Unit - Sepsis.**

Address reprint requests to: Prof. Dr. Med. M. Bauer, Department for Anesthesiology and Intensive Care Medicine, Friedrich-Schiller-University, Erlanger Allee 101, D-07740 Jena, Germany. E-mail: michael.bauer@med.uni-jena.de

Genetic background and course of sepsis

Since Watson and Crick introduced in 1953 the molecular structure for desoxyribonucleic acid (DNA), molecular biology has revolutionized medicine by increasing our understanding of the pathophysiological mechanisms of diseases and by providing novel tools in diagnosing disease due to genetic mutations as well as in assessing genetic risk.[1, 2] In addition, molecular biology and in particular recombinant DNA-technology is developing increasing impact on contemporary therapy. In the critically ill, septic patient, molecular biology has made its way into diagnostic procedures as well as into therapy. Aim of this article is to give an overview of how molecular biology might help to explain the wide variations in the individual response to infection that has long

puzzled clinicians and to summarize new molecular techniques of evaluation and treatment of septic patients.

In 1988 Sorensen *et al.* challenged the understanding of sepsis fundamentally by pointing out, that the genetic influence on the risk to die from an infectious cause is not negligible. In their study the authors followed 960 families that included children born during the period 1924 through 1926 who were placed early in life with adoptive parents. The risk of dying from all causes or from specific groups of diseases between the age of 16 and 58 years for adoptees with a biologic or adoptive parent, who died of the same cause before, was compared. The death of a biological parent before the age of 50 resulted in relative risks of death in the adoptees of 1.71 for all causes, 1.98 for natural causes, 1.19 for cancer, 4.52 for cardiovascular and cerebrovascular causes and 5.81 for infections. The authors concluded that premature death in adults in general has a powerful genetic background, especially death caused by infections.[3] At first sight it seems astonishing that the risk to die from an infection is more influenced by the genetic background than the risk to die from cancer, but it is now obvious that the clinical picture of septic patients is not determined by microbiological species or site of infection alone, but also by the host response.

Malaria was the first infectious disease to be extensively studied in different African populations for genetic variability and susceptibility to disease. Many polymorphisms (see Glossary for definition) conferring susceptibility or resistance have been identified.[4] Today it is well established that polymorphisms in human leukocyte antigen genes correlate with susceptibility to infections including not only malaria but also tuberculosis, hepatitis B and HIV.[5] In African populations, the rarer A variant in the tumor necrosis factor-α (TNF-α) promoter at position -308 was reported to be associated with improved survival in cases of severe cerebral malaria when compared with the more common G allele.[6] The same A variant in the *TNFA* gene, however, was shown to be linked with an impaired survival in patients with septic shock.[7]

These facts lead to the conclusion that the pathogenetic concepts of sepsis may be oversimplified as the result of exacerbated inflammatory responses, since pathogenesis involves multiple factors that interact in a sequence of events from recognition of pathogen-associated molecular patterns (PAMP) towards inflammation or immunparalysis. In today's center of interest, interactions of epithelium, PAMP, proinflammatory mediators, anti-inflammatory signaling, endothelium and leukocytes have been addressed in preclinical and clinical studies.[8] Each interaction again is based upon the interplay of multiple genes and might be potentially influenced by numerous genetic variations. For diagnostic reasons, it is highly interesting to see that a differential gene expression pattern in RNA obtained from blood leukocytes was associated with pathophysiological processes subsequently paralleled by clinical sequelae of systemic inflammatory response syndrome and multiple organ dysfunction syndrome (MODS). Interestingly, some of the differentially expressed genes were even present already prior to surgery.[9, 10]

For a long time clinicians were puzzled by the wide variation of individual response to infection. Today it is well accepted that susceptibility to and outcome of infectious diseases depend on polymorphisms controlling transcription efficiency *e.g.* of *TNFA, IL1B, IL6, PAI1* or within the toll-like receptor genes. The best characterized genetic variation in the human genome as it relates to disease activity is the single nucleotide polymorphism (SNP), which is a mostly biallelic variation of a single base. The analysis of SNPs and their association with the phenotype provides a powerful tool to assess impact of genetic factors in critical care. With assistance of a combined method using gel-based sequencing and high-density variation-detection DNA chips, the characterization of human diversity at the nucleotide level has been demonstrated and improved the understanding of molecular mechanisms in sepsis. Trial product genotyping chips were developed that allow simultaneous genotyping of 500 to 50 000 SNPs and might be in routine use soon and may along with diagnostic

TABLE I.—*Genes and their variants involved in modern concepts of pathophysiology of sepsis.*

Gene activity influenced by described polymorphisms	Clinical significance
Tumor necrosis factor alpha (TNFα) (TNF, OMIM 191160)	May influence mortality in patients with pneumonia [18]
Tumor necrosis factor beta (TNFβ) (LTA, OMIM 153440)	Development of severe posttraumatic sepsis [19] Associated with susceptibility to septic shock and death due to septic shock [7, 20, 21]
Interferon-gamma (IFNγ) (IFNG, OMIM 147570)	TNF_{β} NcoI polymorphism has been associated with patients' nonsurvival [12] Associated with the development of severe posttraumatic sepsis [22] Increased risk for developing sepsis after traumatic injury in patients homozygoteneous for the D allele (DD) [23]
Interleukin-1 alpha (IL1α) (IL1A, OMIM 147760)	Moderates immune responses and illness severity after respiratory syncytial virus infection [24]
Interleukin-6 (IL6, OMIM 147620)	IL-1raA2/IL-1RN2 may contribute to susceptibility to sepsis [12, 25]
Interleukin-10 (IL10, OMIM 124092)	Reduced mortality in patients suffering from acute respiratory distress syndrome (ARDS),[13] systemic inflammatory response syndrome (SIRS),[14] sepsis [15] May influence the severity of illness in patients with pneumonia [26]
Plasminogenaktivatorinhibitor 1 (SERPINE 1, PAI1, OMIM 173360)	Lower stimulation of interleukin-10 release associated with increased mortality in sepsis [26, 27]
Factor V Leiden (F5, OMIM 227400)	PAI-1 4G allele is associated with high concentrations of PAI-1 in plasma and a poor survival rate after severe trauma [17]
Toll-like receptor 2 (TLR2, OMIM 603028)	Most common genetic risk factor for thrombosis [28] Reducion of bleeding after cardiac surgery [16]
Toll-like receptor 4 (TLR4, OMIM 603030)	May predispose individuals to life-threatening bacterial infections [29] Higher incidence of gram-negative infection [30]
Lipopolysaccharide-binding protein (LPB, OMIM 151990)	May predispose individuals to develop septic shock with gram-negative microorganisms [31] Associated with death due to sepsis in patients homozygeneous for Gly98 and/or Leu436 LBP alleles [32]
Mannose-binding lectin (MBL2, OMIM 154545)	Genetic variants of MBL might account for up to 1/3 of all cases of meningococcal disease [33]
Angiotensin-converting enzyme (ACE, OMIM 106180)	ACE D allele is an independent risk factor for pneumonia in the elderly [34] and acute respiratory distress syndrome (ARDS) [35] ACE DD is associated with increased illness severity in meningococcal disease in children [36]
Fcgamma receptor (FCGR2A, OMIM 146790)	Influences susceptibility or course of meningococcal disease [37-39] FcgammaRIIA-H131 polymorphism may be protective for pneumococcal sepsis [40] May contribute to generalized infections caused by encapsulated bacteria [41]

microarrays help to describe the individual problem of the critically ill patient substantially better compared to current biomarkers. [11] A small but functional significant selection of the currently known 1 800 000 SNPs is listed in Table I.[17, 12-41]

The impact of gene polymorphisms on the understanding of sepsis but also the dilemma of contradictory results are exemplarily reflected in the numerous studies addressing the *TNFA* gene locus.

TNF-α is a principal inflammatory mediator in sepsis regulating many downstream cytokines such as IL-6 and IL-10. Specific polymorphisms of the genes encoding TNF-α and TNF-β have been suggested to correlate with higher mortality in septic shock, but a study in 44 patients with systemic inflammatory response syndrome indicated, no difference in the incidence of sepsis, septic shock and mortality between patients bearing the various alleles.[42] A study by Gordon *et al.* investigated in 213 Caucasian patients whether common polymorphisms of the *TNFA* locus and the two receptor genes, *TNFRSF1A* and *TNFRSF1B*, influence circulating levels of encoded proteins, and whether individual polymorphisms or extend-

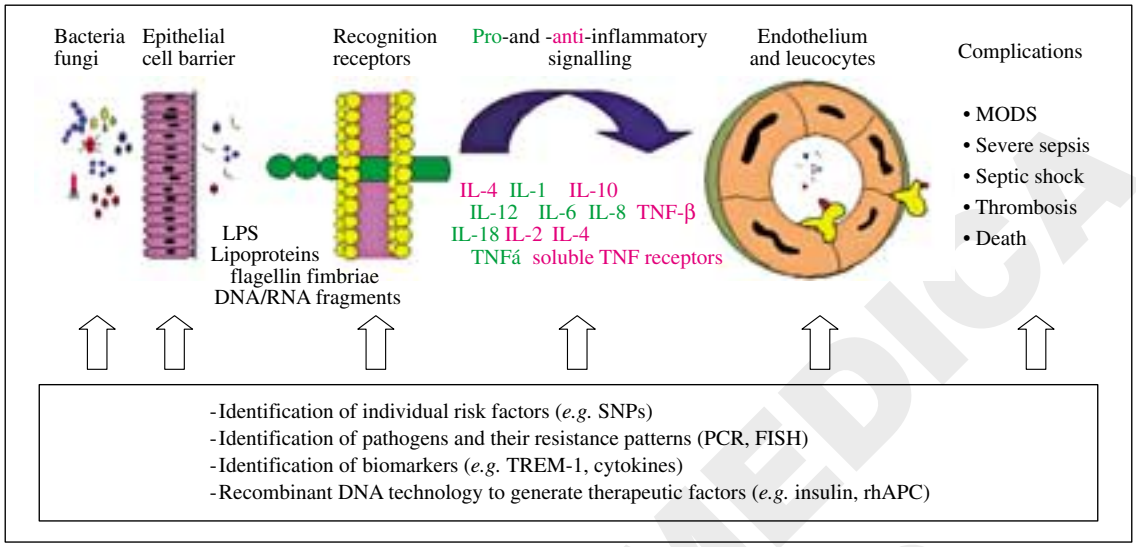

Figure 1.—Overview of modern molecular techniques with possible impact for critical care medicine.

ed haplotypes of these genes are associated with susceptibility, severity of illness or outcome in adult patients with severe sepsis or septic shock. Plasma levels of TNF, sTNFRSF1A and sTNFRSF1B were significantly higher in those who died in ICU compared to those who survived. Thus, these authors demonstrate that there was a positive correlation between increasing soluble receptor levels and organ dysfunction (increasing SOFA score) but they did not find significant association between polymorphisms in TNF-α or TNF-receptors such as TNF-R1 or their haplotypes, and susceptibility to sepsis, illness severity or outcome.[43] In a study in patients of Irish descent, the outcome in patients suffering from meningococcal disease was examined with respect to TNFA polymorphisms and again no correlations between the different polymorphisms and the outcome could be recognized.[44] The study however revealed a correlation of the genetic variability encoding IL-6, IL-10, and an endogenous receptor antagonist for IL-1 *(IL1RN)* with the outcome in meningococcal disease. These controversial results might reflect the protective effects of TNF at the site of infection as opposed to deleterious systemic effects. It is however increasingly evident that beside influences of *e.g.* demographic factors many studies are simply "underpowered" (*i.e.* included not the appropriate numbers of patients) from an epidemiological point of view to link allele frequencies with outcome.

Proinflammatory and anti-inflammatory mediators

Currently known interactions between biomarkers, the interaction with individual risk factors (*e.g.* SNPs), the identification of pathogens and resistance patterns and therapeutical stratagems are summarized in Figure 1.

As already pointed out, TNF-α is playing a central role in the acute inflammatory response and increased levels of TNF-α correlate with severity of disease. Genetic variability at the *TNFA* locus within the MHC locus on chromosome 6 has been well characterized.[45] Nadel *et al.* reported that variations in the *TNFA* promoter are associated with disease severity as children dying from meningococcal disease have significantly higher prevalence for the allele *TNFA*-308A.[46]

The proinflammatory cytokine IL-1 increases plasma concentrations of platelet activating factor, prostaglandins, and NO and seems to influence the susceptibility to sepsis

depending on genetic variants.[12, 47, 48] IL-6 is another prominent proinflammatory cytokine,[49] triggered by several upstream proinflammatory pathways and is associated with an increased occurrence of shock and death in septic patients.[50] In 1998 a guanine (G) to cytosine (C) polymorphism at -174 in the promoter region of the IL-6 gene, was reported by Fishman *et al.* In the general population the frequency of the *IL6-174C* allele is 40% but is decreased in patients with juvenile rheumatoid arthritis. Healthy subjects, with the *IL6-174C* allele, had significantly lower plasma concentrations of IL-6.[51] Additionally, other polymorphisms in the promoter region of the *IL6* gene may influence *IL6* transcription with complex interactions determined by the haplotype.[52] Polymorphisms of the *IL6* gene are thought to influence the mortality of patients with acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), and sepsis.[13-15]

IL-10, a prototypic anti-inflammatory cytokine produced by T-helper type lymphocytes, decreases the production of the proinflammatory cytokines IL-1, IL-6, and TNF-α.[53] In patients with chest and abdominal trauma the ratio between IL-6 and -10, measured in the first 4 days after trauma, correlates with severity of trauma. An unfavorable shift of the IL-6/IL-10 ratio predicts a poor outcome.[54] In patients suffering from hepatitis C, polymorphisms of the *IL10* promoter region, *IL10-592A* and *IL10-819T* on chromosome 1, are associated with lower IL-10 production and a better response to interferon-A therapy than the patients with other haplotypes.[55] To date, these polymorphisms have not been finally evaluated in sepsis. However, these examples underline the potential of immunomodulatory therapies based on a certain genetic risk profile.

Similarly the activation of the complement system via the classic pathway, the lectin pathway or the alternative pathway depends on multiple triggering of genes. The evaluation and modulation of these genes might be of special interest, since in rats the survival rate after cecal ligation and puncture could be significantly improved after treatment with IgG antibodies against C5a.[56]

The balance between protective and destructive effects of the immune system is highlighted by the description of a novel function of caspase-12. Up to now, the enzyme was only considered to play an essential role in modulating programmed cell death [57] and functions to that effect during sepsis.[58] Saleh *et al.* demonstrated a variation in the synthesis of caspase-12 of either a truncated protein or a full length pro-enzyme. The different variants showed marginal differences in their apoptotic regulatory response to various stimuli. In contrast, expression of the long variant resulted in the production of fewer cytokines after endotoxin challenge than did the shorter variant, suggesting that caspase-12 has a role in modulating endotoxin responsiveness and cytokine release.[59]

As a novel therapeutic strategy, the direct modulation of transcriptional activity of genes is suggested by Matsuda *et al.*: since nuclear factor-kappa B (NF-κB) plays a key role in regulating expression of several genes involved in the pathophysiology of endotoxic shock, synthetic double-stranded DNA with high affinity for the NF-κB binding site was introduced into mice 1 h before intravenous injection of *Escherichia coli* endotoxin. Alterations of lung vascular permeability, arterial pO_2, pCO_2, and pH induced by endotoxin were significantly inhibited by *in-vivo* competition of NF-κB gene using consensus sequences in comparison to random mixed oligonucleotides.[60] As described above, similar therapeutic effects could be demonstrated for gene therapy targets such as TNF, rho-kinase and MYPT1.

The epithelium as an entrance for infection

Genetic variations lead to fundamental, individual changes in cell barrier, signal transduction and cellular defense mechanisms. From the moment of invasion of microbes into the host until development of eventual organ failure, regulation of specific genes plays an important role and influences the course and outcome in critically ill patients.

This will be briefly exemplified for each crucial step in this defense mechanisms.

In CD95- or CD95 ligand-deficient mice, bacteria-mediated epithelial-cell apoptosis could contribute to immune defense via activation of the Fas/Fas ligand system.[61] The adenoviral mediated transgenic expression of TNF-α within the lung in mice increased survival after intratracheal challenge with *Pseudomonas aeruginosa* from 25% in animals receiving control vector to 91% in animals administered recombinant murine TNF adenoviral vector. This was paralleled by enhanced lung bacterial clearance and proinflammatory cytokine expression, as well as enhanced alveolar macrophages phagocytic activity and cytokine expression when alveolar cells were subjected to culture ex-vivo. These observations suggest that intrapulmonary immune stimulation with TNF-α can reverse sepsis-induced impairment of antibacterial host defenses.[62] Birukova *et al*. suggest rho-kinase and MYPT1, critical for prevention of thrombin-induced endothelial cell barrier disruption, associated with dramatic gap formation, cytoskeletal reorganization and pulmonary edema in acute lung injury, as a potential gene therapy target.[63] These examples demonstrate that alterations of the epithelial tight junctions and thus barrier function and/or associated clearance mechanisms might promote bacterial translocation and sepsis. This is of special interest since a common cause of sepsis or in general terms of the systemic inflammatory response in the critically ill, mechanically ventilated patients seems to derive from infections of the lungs or from translocation of bacteria from the intestinal or bronchial tract into blood or the lymphatic system.

Recognition receptors

Surface molecules, such as endotoxin (lipopolysaccharide), lipoproteins, outermembrane proteins flagellin, fimbriae, peptidoglycan, peptidoglycan-associated lipoprotein, and lipoteichoic acid and internal motifs released during bacterial lysis, such as heatshock proteins and DNA as well as RNA fragments from pathogenic, non-pathogenic, and commensal bacteria are recognized in the host by specific PAMP. These receptors belong to the family of toll-like receptors and are characterized by an extracellular leucine-rich repeat domain and a cytoplasmic toll-interleukin-1 receptor domain. Currently more than 10 toll-like receptors have been shown to contribute to defense against microbes and the list of their ligands is growing.[8]

In addition, NOD proteins (NOD1, NOD2) serve as intracellular receptors for bacterial products in monocytes and transduce signals leading to NF-κB activation and are considered to contribute to localized cell death at the site of pathogen invasion. Structurally, the proteins are functionally characterized by N-terminal caspase recruitment domain, a centrally located nucleotide-binding domain, and a C-terminal regulatory domain with leucine-rich repeats. A panel of SNP in the NOD2 gene were determined to be associated with an increased susceptibility to Crohn disease, a disorder characterized by episodic intestinal inflammation with epithelioid granulomas.[64, 65] Patients with double-dose mutations were characterized by a younger age at onset, a more frequent stricturing phenotype, and a less frequent colonic involvement than in those patients who had no mutation.

Recently it has been demonstrated, that the cytosolic protein efficiently detects bacterial peptidoglycan with subsequent NF-κB activation as a positive regulator and IL-1β secretion.[66] A quantitative parameterization of this response, computed from patients with different genotypes, was predictive of several variable manifestations of Crohn disease. In infants with very low birth weight, an insertion (NOD2-3020insC) was associated with the development of neonatal sepsis, a higher rate of positive blood culture (33% *vs* 14%), and an increased susceptibility for repeated septic episodes.[67]

Endothelium and leukocytes

Activated endothelial cells functionally located between blood stream and tissue

promote adhesion of leucocytes, which can then migrate into inflamed tissue. Experiments with ICAM-1 knockout mice [68] or animals treated with adhesion molecule-specific antibodies [69] suggest that adhesion molecules expressed on leucocytes or endothelial cells (*i.e.* lymphocyte function associated antigen 1, intercellular adhesion molecule 1, endothelial leukocyte adhesion molecule 1, L-selectin, and P-selectin) might contribute to endorgan damage even though adhesion-molecule blockade worsened cardiovascular and metabolic functions in several models of sepsis. This may reflect that migration of leukocytes from the blood stream to inflamed tissues [70] is associated with the release of numerous proteases that play a pivotal role in combating infections. For example, compared with controls, mice that have a knockout of the neutrophil-elastase gene are more susceptible to sepsis and death after intraperitoneal infection with gram-negative, however, not gram-positive bacteria.[71]

Molecular biology to diagnose bacterial infections

The blood culture as gold standard for bacterial diagnostics is slow and lacks sensitivity especially when the patient has previously received antibiotics or in the presence of fastidious organisms, such as *Mycobacteria, Actinomyces, Nocardia* or *Borrelia burgdorferi.* Improved methods have therefore emerged for the detection of bacteria and fungi *in-vitro* and in the blood stream such as pathogen-specific or broad-range polymerase chain reaction (PCR) assays and fluorescence *in situ* hybridization (FISH) exhibiting promising results.[72] They are expected eventually to replace the current conventional microbiological techniques for detection of bloodstream infections. The advantages of these techniques are reduced time requirements to obtain results, their sensitivity and specificity even when only small sizes of samples can be acquired (*e.g.* liquor) and their advantage in detecting chains of infections.[73, 74] The amplification of *e.g.* 16S-ribosomal bacterial RNA sequences is conceptually appealing for rapid and sensitive diagnosis of infection [75] which is essential for an effective goal directed anti infectious therapy.

Additionally, not only the gram stain classification of bacteria but also the resistance/susceptibility pattern of *e.g. Mycobacterium tuberculosis* or methicillin-resistant *Staphylococcus aureus* can be determined in a rapid and specific manner by rtPCR for the optimization of the efficacy of antibacterial therapy.[72, 76, 77] The problem deriving from contamination might be emphasized in the mentioned new techniques, since even small numbers of bacteria can be detected.

Coagulation disturbances in sepsis

Many complications in critically ill patients are caused by the imbalance of pro- and anti-inflammatory factors that lead to hypercoagulability or bleeding. The hypercoagulability might, in addition to the well known complications of thrombosis and embolism, lead to an impairment of the microcirculation which again might be responsible for the development of MODS or septic shock. In plasma of patients, heterogeneous for G20210A factor II/prothrombin-SNP, elevated concentrations of thrombin can be measured causing an increased risk for the development of venous thrombosis.[78] In 1994 factor-V-Leiden polymorphism (A1691G), the most common genetic risk factor for thrombosis, was identified. However patients with factor-V-Leiden polymorphism showed a reduction in bleeding after cardiac surgery.[16] Of clinical relevance is further the plasminogen activator inhibitor (PAI)-1-gene polymorphism. Patients who have the 4G/4G-genotype show an elevation of PAI-1 in plasma leading to a reduction of fibrinolysis, which is of relevance in therapeutic fibrinolytic reopening of occluded vessels. In addition patients having the 4G/4G genotype have a higher risk to develop MODS or septic shock.[17, 79]

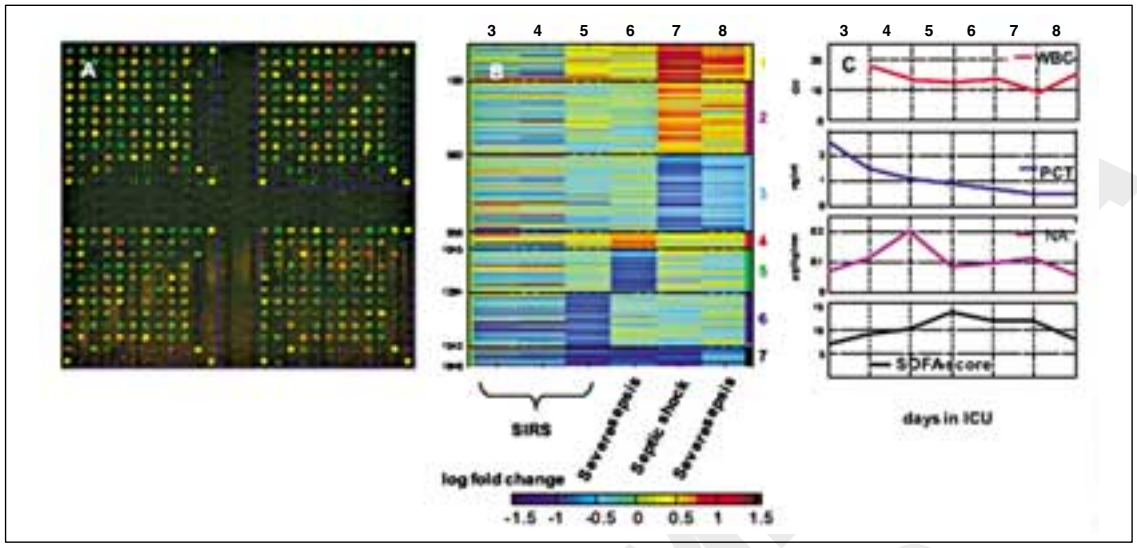

Figure 2.—A) Gene expression analysis using microarray: one dot represents the expression of one specific transcript. B) Representative gene expression pattern of a patient during the timeframe of 6 days during the course of sepsis after CABG (day 1). Changes in gene expression profiles (red increase, blue decrease) correspond to transition from disease severity classes, such as SIRS (day 3 to day 5) to severe sepsis (day 6 and 8) and septic shock (day 7). C) Corresponding changes of current clinical markers in the same patient: WBC (red), procalcitonin (PCT, blue), catecholamine consumption (pink) and SOFA-score (black) representing the severity of illness. [Illustration kindly provided by Stefan Russwurm, SIRS-Lab GmbH, Jena, Germany].

Molecular biology to identify patients at risk

There is a large body of evidence in the literature on putative mediators and markers of sepsis as well as polymorphic variants in the encoding genes. Nevertheless, studies concerning the application of currently known mediators or the blockade of recognition receptors were mostly disappointing.

Worth mentioning however is that the evaluation of IL-6, IL-8 and TREM-1 triggering receptors has become a standard procedure in many ICUs to quantify the success of antimicrobial therapy.[80] Besides the important clinical signs, the diagnosis of sepsis has been based on biomarkers such as white blood count and lactate levels. Procalcitonin has been put into focus as a parameter to differentiate SIRS and sepsis. There is no ideal single sepsis marker available yet. Recent evidence suggests that cell-free plasma DNA has potential use as a prognostic marker in critically ill patients representing a predictor of death.[81] The array technique finally might fulfil the demands expected from an ideal sepsis marker: high sensitivity and specificity for sepsis, possibility to differentiate between infectious and non infectious causes of inflammation, organ dysfunction and shock, presence at the onset or even before the appearance of clinical signs of sepsis and indication of severity and course of sepsis. Regardless of the heterogeneity of critically ill patients, a striking correlation between the conventional diagnostic classification and the novel approach with micro array technique was observed.[10, 82] (Figure 2). Thus, microarray technique might on one hand lead to a reduction of unnecessary therapy with antibiotics, but it might on the other hand provide an early trigger to start a sufficient chemotherapeutic offensive. Microarray technique introduced 1995,[83] is still struggling as a standardized analytic procedure that can be utilized in routine at acceptable costs. It can be speculated however, that the pangenomic analysis by microarrays will open up new dimensions in research of the pathophysiology of sepsis in preclinical and clinical trials before routinely used in ICUs.[84]

Beyond a general trigger the genetical analysis might also influence the quality of therapy, since different septic sources (peritonitis, pneumonia, burn or musculo-skeletal infections) may result in different mRNA patterns. The rtPCR and array technique turned out to be revolutionary and highly useful tools to monitor the immune response. Studies have demonstrated a very high variability in inflammatory mediator expression in septic patients compared to healthy volunteers.

This suggests that any future immune-modulatory therapy may need to be individualized with respect to the patient's requirements as monitored by rt-PCR and/or array technique. Different sources of sepsis and individual genetic varieties in patients may result in markedly different activation patterns and thus might require different therapeutic actions.[85, 86]

Pharmacogenetics investigate the impact of genetic background on pharmacokinetics and pharmacodynamics.[87-90] Mutations influencing transport proteins, excretion, receptors or enzymes involved in metabolism are the target of investigations, ultimately aiming at developing individually adjusted therapy which guarantee maximal efficacy in combination with a minimum of side effects. The new emerging scientific and commercially important field is described by the term "theragnostics".

Since Watson and Crick described the molecular structure of DNA, the impressive progress in molecular biology techniques has not only revolutionized the understanding of the pathophysiology of critically ill patients suffering from sepsis but has also enabled development of novel methods improving microbiological diagnostics and individual assessment of the patient's state of defense enabling new patient-orientated approaches ("theragnostics"). A careful application of these novel and promising molecular biologic strategies must remain the first duty of scientists and clinicians. However, individualized immune-modulatory gene therapy holds promise to revolutionize also therapeutic options not only in critical care medicine.

Riassunto

Biologia molecolare in un'Unità di Terapia Intensiva: dalla comprensione al trattamento della sepsi

Sempre più dati suggeriscono che oltre a fattori ben noti, quali la virulenza dei patogeni o la sede di infezione, le differenze individuali nella manifestazione della malattia sono il risultato di una predisposizione genetica del paziente ricoverato in un'Unità di Terapia Intensiva. Fattori genetici specifici potrebbero non solo predire il rischio di acquisire una grave infezione ma anche di sviluppare un deficit d'organo o, in definitiva, di morire. Di conseguenza, l'avvento delle tecniche molecolari, consentendo lo screening per un'ampia varietà di fattori genetici, quali i polimorfismi di un singolo nucleotide nei geni che controllano l'espressione di importanti sistemi di mediatori nei pazienti, così come il loro preciso bersaglio nei modelli animali di sepsi, stanno rivoluzionando la comprensione della patofisiologia del paziente criticamente ammalato. Gli strumenti della biologia molecolare sfidano i test diagnostici più avanzati, come l'emocoltura, dato che non solo aumentano la sensibilità ma riducono drammaticamente il tempo necessario per identificare i patogeni e i loro quadri di resistenza. Allo stesso modo, la conoscenza dei fattori genetici potrebbe in un prossimo futuro aiutare a identificare i pazienti a rischio, ad esempio quelli con un'elevata probabilità di sviluppare un deficit d'organo, o a guidare gli interventi terapeutici, in particolare quelli che riguardano le terapie che richiedono molte risorse e/o quelle costose ("teragnostici"). Mentre le opzioni terapeutiche della medicina intensiva molecolare, come le cellule staminali nel trattamento dell'insufficienza d'organo o il trasferimento genico terapeutico, saranno possibili in futuro e potrebbero divenire un'alternativa, la tecnologia del DNA ricombinante ha già un suo ruolo ben definito nella produzione di proteine umane ricombinanti, dall'insulina alla proteina C attivata.

Parole chiave: Biologia molecolare - Unità di terapia intensiva - Sepsi.

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Glossary

Adhesion molecule

A diverse family of extracellular and cell surface glycoproteins involved in cell-cell and cell-extracellular matrix adhesion, recognition, activation, and migration.

Allele

One of several variants of a polymorphic gene occurring at the same locus on homologous chromosomes and governing the same biochemical and developmental process.

Biomarker

Measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health - and physiology related assessments, such as disease risk. Biomarkers reflect a variety of disease characteristics, including the level of exposure to an environmental or genetic trigger, an element of the disease process itself, an intermediate stage between exposure and disease onset, or an independent factor associated with the disease state but not causative of pathogenesis. Depending on the specific characteristic, biomarkers can be used to identify the risk of developing an illness, aid in identifying disease, or predict future disease course, including response to therapy.

CD antigen

Cluster of differentiation designation assigned to leukocyte cell surface molecules which are identified by a given group of monoclonal antibodies.

Chemokines

A family of structurally-related cytokines which selectively induce chemotaxis and activation of leukocytes. They also play important roles in lymphoid organ development, cell compartmentalization within lymphoid tissues, Th1/Th2 development, angiogenesis and wound healing.

Cytokines Low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells.

Fas/Fas Ligand system

A member of the TNF receptor gene family. Engagement of Fas (CD95) on the surface of the cell by the Fas ligand (CD178) present on cytotoxic cells, can trigger apoptosis in the Fas-bearing target cell.

Genomics

The study of the functions and interactions of all the genes in the genome, including their interactions with environmental factors.

Haplotype

The set of allelic variants present at a given genetic region that are inherited together.

Knockout

The use of homologous genetic recombination in embryonal stem cells to replace a functional gene with a defective copy of the gene. The animals that are produced by this technique can be bred to homozygosity, thus allowing the generation of a null phenotype for that gene product.

Mannose binding protein (MBP)

A member of the collectin family of calcium-dependent lectins, and an acute phase protein. It functions as a stimulator of the classical pathway of complement activation, and as an opsonin for phagocytosis by binding to mannose, a sugar residue usually found in an exposed form only on the surface of microorganisms.

Pathogen-associated molecular pattern (PAMP)

Molecules such as lipopolysaccharide, peptidoglycan, lipoteichoic acids and mannans, which are widely expressed by microbial pathogens as repetitive motifs but are not present on host tissues. They are therefore utilized by the pattern recognition receptors (PRRs) of the immune system to distinguish pathogens from self antigens.

Phenotype

The clinical presentation or expression of a specific gene or genes, environmental factors, or both.

Polymerase chain reaction (PCR)

In-vitro method for expanding large amounts of specific and discrete DNA or RNA fragments of defined length and sequence by the use of small amounts of short oligonucleotide flanking sequences (primers). The essential steps include cycles of thermal denaturation of the double-stranded target molecules, annealing of the primers to their complementary sequences, and extension of the annealed primers by enzymatic synthesis with DNA-polymerase. The reaction is efficient, specific, and extremely sensitive. Uses for the reaction include disease diagnosis, detection of difficult to isolate pathogens, mutation analysis, genetic testing, DNA sequencing, and analyzing evolutionary relationships.

Polymorphism

A term describing loci at which there are 2 or more alleles that are each present at a frequency of at least 1% in a population of animals. The term has been co-opted for use in transmission genetics to describe any locus at which at least 2 alleles are available for use in breeding studies, irrespective of their actual frequencies in natural populations, see also SNP.

Single nucleotide polymorphism (SNP) SNPs are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the least frequent allele has an abundance of 1% or greater. SNPs can occur in coding regions of the genome, in regulatory regions, or, most commonly, in "junk DNA" regions, in which case they are referred to as anonymous SNPs.

Each individual has many single nucleotide polymorphisms that together create a unique DNA sequence. These polymorphisms are highly conserved throughout evolution and within populations and are thus, excellent genotypic markers for both population genetics and individualized therapy.

Theragnostic

Made up of the words 'therapy' and 'diagnostics', key term and current focus of molecular medicine. Tools for screening and monitoring to identify different stages and varying prognoses of a disease, for the development of tests and pharmaceuticals for patients to respond to an individualized specific therapy with respect to specific pathological features. Urgent need for planning and implementation of future clinical trials and the interpretation of results.

Toll-like receptor

Part of the innate immune response team that provide rapid response after they are activated by components of bacteria, viruses and toxins.