■ REVIEW ARTICLE

The genetic predisposition to adverse outcome after trauma

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Technological advances and shorter rescue times have allowed early and effective resuscitation after trauma and brought attention to the host response to injury. Trauma patients are at risk of progressive organ dysfunction from what appears to be an uncontrolled immune response. The availability of improved techniques of molecular diagnosis has allowed investigation of the role of genetic variations in the inflammatory response to post-traumatic complications and particularly to sepsis.

This review examines the current evidence for the genetic predisposition to adverse outcome after trauma. While there is evidence supporting the involvement of different polymorphic variants of genes in determining the post-traumatic course and the development of complications, larger-scale studies are needed to improve the understanding of how genetic variability influences the responses to post-traumatic complications and pharmacotherapy.

An improved understanding of the pathophysiological mechanisms governing the immune response after both traumatic and surgical injury has contributed to our knowledge of the aetiology of post-traumatic complications such as sepsis, the adult respiratory distress syndrome (ARDS) and the multiple organ dysfunction syndrome (MODS).¹ In some respects, the response to trauma resembles an exaggerated activation of the immune system with cell-mediated damage to remote organs, while in other respects immunosuppression predominates.² Many alterations of the immunoinflammatory system have been demonstrated both clinically and experimentally within hours of trauma and haemorrhage, suggesting that a cascade of abnormalities is initiated leading to ARDS and MODS.^{3,4} Several mediators and cellular elements actively participate in this process, co-ordinating the body's endeavour to maintain homeostasis and survival. Among other molecules, cytokines act as mediators regulating these events. They are synthesised and released by leucocytes and endothelial cells after exposure to different chemical and physiological stimuli.²

A number of studies have characterised and quantified the molecular events which occur after trauma. In spite of efforts to predict the outcome for patients accurately, this is, however, not always possible. The expected out-

come can be difficult to determine in some patients and others do better than predicted. In the early 1990s, it was recognised that differences in outcome could be attributed to the existence of biological variations among patients.⁵ The importance of genes as the cause of disease, or as predisposing factors, is now indisputable,⁶⁻⁹ and such biological variation may be highly dependent upon the genetic constitution of the patient. A polymorphism is a naturally occurring variation in the sequence of information on a segment of DNA based on different alleles and located within the gene¹⁰ or in the promoter region.¹¹ Some polymorphisms are mutations located within endonuclease restriction sites^{12,13} whereas others are single nucleotide polymorphisms (SNPs),^{9,11} or consist of insertions or deletions of larger fragments.¹⁰ Specific polymorphic variations may be associated with genetic diseases such as sickle-cell anaemia, thalassemia, Huntington's disease, Friedreich's ataxia, familial Mediterranean fever or Crohn's disease.^{14,15} A polymorphism may also interact with its environment and can be associated with an increase in the risk of osteoporosis¹⁶ and the carcinogenic potential of industrial chemicals, tobacco and alcohol.¹⁷

We have reviewed the current literature regarding the genetic predisposition to adverse outcome after trauma and surgery.

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Polymorphisms

Genetic polymorphisms have been identified and investigated at various levels. Their effects in post-traumatic complications and particularly sepsis are described below. They can be categorised into cytokines, receptors, binding proteins and other inflammation-related autocooids (Fig. 1).

Cytokines

Tumour necrosis factor. This is an integral part of the immune response to infection and is recognised as a central mediator of sepsis and septic shock.¹⁸ The human tumour necrosis factor (TNF) locus consists of two closely-related sequences arranged in tandem on the short arm of chromosome 6 which encodes for two cytokines (TNF- α and TNF- β).¹⁹ Wilson et al¹² indicated the presence of a functional bi-allelic polymorphism in the promoter region of the TNF- α gene at position -308. The two existing alleles are termed TNF1 (-308G) and TNF2 (-308A). The TNF2 allele is associated with greater production of TNF- α .¹³ A bi-allelic polymorphism at position +250 of the TNF- β (lymphotoxin-alpha or LT- α) gene has also been described (TNF- β 1 and TNF- β 2/NcoI).²⁰ The associated functional consequences of these polymorphisms are unclear, but they have been associated with an increased incidence of sepsis and poorer outcome after major trauma, severe burns and sepsis.²¹⁻²³ A study by Stuber et al¹⁸ was unable to demonstrate an association of the -308 variation with increased sepsis and raised the possibility of a linkage between this variation and TNFB2(NcoI). Homozygous patients for the TNFB2 (NcoI) allele have a higher rate of mortality, higher circulating concentrations of TNF- α and greater organ dysfunction than heterozygous patients.^{24,25} One investigation has shown that the observed TNFB2 (coI) associations are only present in male patients with sepsis, and female patients displaying normally distributed genotypes without an adverse outcome.²⁶ However, the influence of polymorphisms of the TNF locus on the susceptibility to, and outcome from, sepsis is not universally accepted.²⁷

Interleukin-1 β . The human Interleukin-1 (IL-1) precursor gene was sequenced in 1985²⁸ and the Interleukin-1 β (IL-1 β) gene is located in chromosome 2q14-21.²⁹ Three polymorphisms of this gene are currently known:³⁰ 1) position 1903 (Threonine (T) $\rightarrow$ Cysteine (C)) in the promotor region; 2) 5810 (Glycine $\rightarrow$ Alanine) in intron 4; and 3) 5887 (C $\rightarrow$ T) in exon 5. They are associated with different restriction of fragment length polymorphism for the endonucleases AluI, BsoFI, and TaqI, respectively.^{30,31} The combination of these three polymorphisms gives rise to the highest information content in comparison with any polymorphism alone.³⁰ The TaqI polymorphism is a 'high-secretor' phenotype which is associated with increased secretory levels of IL-1 β .³¹ However, in one study, the TaqI polymorphism did not seem to influence the outcome

Fig. 1

Diagram showing the central role of cytokines in post-traumatic inflammation. Genetic polymorphisms of the cytokines, cellular wall receptors, binding proteins, and inflammation-related proteins could influence the physiological response and clinical course after trauma.

after sepsis in a series of patients in post-operative surgical intensive care.³²

Interleukin-6. The gene for Interleukin-6 (IL-6) was sequenced in 1986 (chromosome 7p21) and was called B-cell stimulatory factor 2 (BSF-2).³³ Interleukin-6 is a key pro-inflammatory cytokine in the systemic inflammatory response syndrome (SIRS). Increased plasma levels have been associated with an adverse outcome after trauma, and in patients surviving post-traumatic complications significantly lower plasma concentrations of IL-6 have been observed.³⁴ Increased plasma levels of IL-6 have also been associated with severe sepsis, but not with the outcome associated with blunt multiple trauma.³⁵ Polymorphisms of IL-6 have been reported in both the 3 and 5 flanking regions and exon 5.³⁶ Two polymorphisms in the 5 flanking region influence the restriction sites for BglII and SfaNI.³⁷ The latter is located at position 174. A homozygotic constellation of this polymorphism with the C allele at position -174 coincides with significantly decreased serum levels of IL-6 during inflammation, a lower total and differential white blood cell count and increased sensitivity to insulin, compared with carriers of the G allele.³⁸ In the study of Schluter et al,³⁷ patients suffering from severe post-operative sepsis had an improved survival with the genotype GG. Haplotype-based analysis of the C/C/G, G/G/G and G/C/C haplotype clades of IL-6 has shown that they are strongly associated with increased mortality and more organ dysfunction in SIRS.³⁹ Hildebrand et al⁴⁰ observed a significantly higher incidence of the allele IL-6 -174G and the homozygous IL-6 -174G genotype in patients with SIRS. The IL-6 174G/C polymorphism has also been associated with more serious systemic inflammation as judged by scores based on the values of body temperature, heart rate, respiratory rate and white blood cell count.⁴¹

Interleukin-10. The interleukin-10 (IL-10) gene has been mapped to chromosome 1q31-32, and a number of

polymorphisms in the promoter region have been characterised.⁴² Three at -1082(A/G), -819(C/T), -592(C/A) upstream from the transcription start site⁴³ have been described, as well as two additional microsatellite (CA) repeats, termed IL-10G and IL-10R, located at -1151 and -3978, respectively.⁴⁴ Variable associations have been reported between IL-10 polymorphisms, IL-10 production and autoimmune diseases.⁴⁵ Recent data have shown the influence of different SNPs of the IL-10 promoter during the development of sepsis, but these findings are somewhat controversial.^{46,47}

It is now established that IL-10 SNPs are completely (-9 to -592) or strongly linked (-1082 to -819 and -592). In Caucasians, only three haplotypes have been found: GCC (G at position -1082, C at position -819 and C at -592), ACC and ATA.⁴³ These have been associated with high (GCC), intermediate (ACC) and low (ATA) production of IL-10.⁴⁸ The highest levels of IL-10 were produced in homozygous GCC/GCC haplotypes.^{48,49} The -592A allele has a lower transcriptional activity, with decreased secretion of IL-10 seen *in vitro*.^{43,46,48} Patients carrying the genotype -597AC have significantly higher overall scores of organ dysfunction and those with the genotype -592AC have a 3.3-fold increase in the risk of developing multiple organ dysfunction, but further studies with greater power are required to support this finding.⁵⁰

Eskdale et al⁵¹ found that stimulation of human blood cultures with bacterial lipopolysaccharide demonstrated a genetically-determined large interindividual variation in the secretion of IL-10 (> 70%). They observed that those haplotypes containing the allele IL-10.R3 were associated with lower secretion of IL-10 than those containing any other IL-10.R allele. The haplotype IL-10.R2/IL-10.G14 was associated with the highest secretion of IL-10 overall, while haplotype IL-10.R3/IL10.G7 was associated with the lowest. Similar results have been obtained for the dinucleotide repeat at -592 bp.⁴⁶

Interleukin-18. Stassen et al⁵² extracted DNA from the peripheral leucocytes of trauma patients with an injury severity score (ISS) ≤ 16. Two SNPs (-607bp and -137bp) were amplified; each had two alleles and three genotypes. Individually, each SNP had no direct correlation with the patient's genotype and the development of infection, but when the -607bp CA genotype was combined with the -137bp GC genotype (CA/GC), only four patients (27%) developed sepsis.⁵² This suggested that IL-18 genetic promoter polymorphisms may determine the development of post-injury sepsis.

Interferon-γ. Stassen et al⁵³ evaluated the relationship between polymorphisms in the first intron of the interleukin-γ (IFN-γ) gene and the development of sepsis after trauma (ISS ≤ 16). The ISS, race, age and gender distribution were similar for both the septic and non-septic groups. Six alleles and ten genotypes were identified. Patients who were septic had a 62% chance of having a D allele (Student's *t*-test, *p* = 0.06), whereas they had only a

29% chance of having a C allele. Homozygotes for allele D (DD) were the most likely to become septic (65%)⁵³ and had an increased chance of developing sepsis after traumatic injury compared with other allelic combinations.⁵³

Receptors. Several gene polymorphism receptors have been discovered indicating susceptibility to infection and sepsis. These include the IL-1RN gene polymorphism,⁵⁴ the interferon-γ receptor 1,^{54,55} the toll-like receptor 4⁵⁶ and the toll-like receptor 2.⁵⁷

Binding proteins

The role of polymorphisms of a number of binding proteins has also been investigated extensively. Lipopolysaccharide binding protein,⁵⁸ mannose-binding lectin,⁵⁷ bactericidal permeability increasing protein⁵⁹ and CD14⁶⁰ have all been shown to have an influence on the development of sepsis and mortality.

Other inflammation-related proteins

Several other proteins have been found to be associated with post-traumatic complications and direct pulmonary injury including surfactant protein-B,⁶¹ plasminogen activator inhibitor type-1 glycoprotein,⁶² coagulation factor V,⁶³ tissue factor,⁶⁴ thrombomodulin,⁶⁵ vascular endothelium growth factor⁶⁶ and renin-angiotensin.⁶⁷ Recently, the role of lipid mediators has also been suggested postulating the existence of a link between lipoproteins and apolipoproteins (APOE), general host inflammatory responses and the outcome after sepsis.⁶⁸⁻⁷¹

Some studies have suggested that APOE-deficient animals display impaired immune responses and increased susceptibility to endotoxaemia, and bacterial and fungal infections.^{72,73} Exogenous administration of APOE or APOE-mimetic peptides in animals has been found to suppress local as well as systematic inflammatory responses⁷⁴ and to decrease mortality.⁷⁵ Independent of lipoprotein transport, APOE can have a dual pro- or anti-inflammatory effect⁷⁶ which could be explained by cell-specific differences in APOE isoform binding.⁷⁷ In an elective surgical series studied by Moretti et al,⁷⁸ the presence of the APOE ε3 allelic variant of the APOE gene was associated with a decreased incidence of severe sepsis and a shorter length of stay in intensive care. A poliprotein genotypes were not associated with the duration of mechanical ventilation or mortality.⁷⁸

Discussion

Technological advances and shorter rescue times have improved early and effective resuscitation and emphasised the treatment of the host response to injury. Trauma patients are at risk of progressive organ dysfunction from what appears to be an uncontrolled immune response. The availability of improved molecular diagnostic techniques provides 'disease-gene association' studies to investigate the role of genetic variations in the inflammatory response to post-traumatic complications and partic-

ularly to sepsis. However, the characterisation of the immune response to surgical and accidental trauma remains incomplete and the list of candidate genes affecting or influencing a disease process is not fully known. The homeostatic mechanisms are regulated by complex pathways and there is substantial overlap between the stimulatory effect of molecular mediators and the cellular responses. Several molecules also exert a dual regulatory effect, complicating matters further.

Despite these problems, specific strategies of treatment with modifiers of the biological response have been studied in clinical trials, but so far no clear benefit in outcome has been found.⁷⁹ There may be several reasons for this. First, it has become clear that it is difficult to identify patients who may benefit from this type of therapy and the aetiology of some of the complications may be different. For example, pulmonary dysfunction can be the result of an injury causing contusion of the lung or to the gas exchange being altered as a result of an infectious stimulus, as in pneumonia. Hence, different molecules may be responsible for triggering the exaggerated immunoinflammatory responses. Secondly, there may be some critical delay between the onset of complications and the initiation of treatment. Knowledge of the timing of expression of specific candidate genes is crucial in order to deliver at the appropriate time any pharmacological agents which could exert a positive physiological effect. Thirdly, there may be cofactors such as comorbidities, or drug effects which slow down the cell turnover that are currently unrecognised and may interfere with the desired effect of the gene expression. These obstacles could be overcome by early identification of these factors. Functional polymorphisms represent naturally occurring variations in the sequence of genetic information on a segment of DNA. They provide a potential mechanism whereby profiles of risk factors or variations may be identified. In this review, the available evidence has been categorised into cytokines, surface receptors, binding proteins and other inflammation-related autoids. The genetic control of the release or upregulation of the inflammatory mediators or cytokines described may determine the degree and duration of the pro- or anti-inflammatory response and the outcome of infection or other post-traumatic complication.⁸⁰ For instance, IL- β has been strongly implicated in the pathophysiology of sepsis and septic shock and its gene polymorphisms may serve as candidate markers for patients at high risk of developing sepsis.^{30,81} However, together with IL-1Ra, its contribution to severity and outcome is unclear.³⁰ Tumour necrosis factor- α also has been extensively studied and the importance of the TNF2 and TNFB2 alleles in the outcome of sepsis is most evident from the studies investigating pneumococcal and meningococcal disease, in sepsis in surgical patients or after blunt trauma.^{30,82} Similarly, functional SNP in the CD14 promoter increases sCD14 resulting in decreased levels of IgE in healthy children⁷⁹ and is strongly associated with an

increased risk of death from septic shock.⁵⁷ Although other SNPs have been found to be associated with complications, it is believed that most have no impact on biological function. The inflammatory response operates both in series and in parallel in a highly complex network, with a substantial degree of redundancy. Hence, a single functional polymorphism is unlikely to influence the overall inflammatory response in more than a fraction of the patients.⁸³ Large groups of patients are required to demonstrate a significant, yet small, association between an SNP and a clinical event.⁸³ When assessments of SNPs are used as a tool to predict the clinical course of patients in intensive care, ideally a large number of SNPs should be examined simultaneously. This method would identify influential combinations of SNPs and would have a higher predictive power.⁸³

The number of candidate genes and polymorphisms under investigation is rising.^{79,80} It is important to appreciate that when determining the presence and association of a functional polymorphism with a post-traumatic complication, several criteria should be taken into account. The ethnic distribution and the clinical heterogeneity of the population studied has to be taken into consideration.^{21,79,80} Family-based studies could reveal possible underlying genes involved in the disease process. The power of the study also has to be sufficient and well controlled in order to explore a specific hypothesis. Studies investigating weakly penetrant genes have the potential to yield false-negative results if underpowered. One of the pertinent clinical problems is that it is currently difficult to perform a power analysis in order to determine the required number of patients for a certain study. Depending on how many genes or polymorphisms are involved, the degree of expression of certain diseases or complications may vary. In particular, the question as to whether other polymorphisms may be involved should be considered, as well as the pattern of occurrence of particular disease-associated haplotypes in the patients under investigation. Such polymorphisms may be the actual cause of any differences detected. It is possible that the investigated polymorphism may be an epiphenomenon or in linkage disequilibrium with a functional polymorphism,^{13,79,84,85} rather than the underlying cause. If differences in disease outcome are linked to one or more genetic polymorphisms, a subsequent study may have to be performed in another cohort, just to assess this issue. This cohort will have to show a similar linkage of genes to outcome. In this respect, it is important to notice that not all clinical studies have shown a link between a genetic variance and a complication. This applies for the susceptibility to infection or survival in septic patients⁸⁶ and the failure to find a difference between severe sepsis and control groups.¹⁸

Therefore, unlike investigations assessing clinical risk factors for certain diseases, the number of studies required to determine the influence of a certain polymorphism may

Table I. Validation criteria for studying genetic polymorphisms

Overall genetic constellation of the population
Power of the study
Other genetic associations/epiphenomena
Family genetics
Reproducibility in a second cohort
Prospective or retrospective study

be much higher. In several countries, multicentre studies have been initiated which may clarify some of these questions.

Another important factor for consideration is whether the presence of the polymorphism is related to the clinical course rather than to the disease under investigation. Majetschak et al²² described this finding in patients who developed post-traumatic sepsis. Hence, even if a result is regarded as positive, the clinical significance may need to be clarified.

The investigation of genetic polymorphisms should adhere to the basic rules listed in Table I. Currently, a number of studies has suggested the influence of specific polymorphic variants of important genes in post-traumatic complications and in particular in sepsis. However, not all have fulfilled the criteria mentioned in Table I. It is uncertain whether studies on genetic association are consistently reproducible. Factors affecting reproducibility include the ethnic admixture, variable linkage disequilibrium and genotype misclassification.^{3,80} Larger-scale studies adhering to specific criteria of genetic association are required.

The aetiology and understanding of the development of complications after trauma have been the focus of many research groups. Several studies have been undertaken in patients in intensive-care units or while in hospital. Most have looked primarily at the link between the inflammatory system and the systemic complications, although many other cascade mechanisms, such as the haemostatic response, are also known to play a major role. With the ongoing advances in diagnostics and in molecular biology all the known pathways of interest will eventually be explored.

Future research should focus on a broad array of genes. Single nucleotide polymorphism genotyping assays are currently the most favourable option to categorise patients. These methods have been integrated in the multicentre studies addressed above. In the future genetic information obtained at presentation will help to predict outcome. Early identification of patients at risk will allow direct interventions which modify the biological response to improve the rates of morbidity and mortality. This will enable clinicians to perform early, goal-directed therapy using conventional drugs, or even by creating specific 'genetic' pharmacological agents.

An early, goal-directed approach is able to improve outcome. Low-dose steroid supplementation, blood glucose control and activated protein C (known to have anti-thrombotic, anti-inflammatory and profibrinolytic properties in severe sepsis) therapy appear to be associated with an improved outcome after sepsis.⁸⁷⁻⁸⁹

Further advances will improve the understanding of how genetic variability influences responses to complications and pharmacotherapy.

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