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The extracellular actin scavenger system in trauma and major surgery

Clinical and experimental studies

Benny Dahl, MD, PhD

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"Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent."

Calvin Coolidge 1872–1933
Vice President 1921–1923
President 1923–1929

Contents

List of Papers

This thesis is based on the following publications:

I. Dahl B, Schiødt F, Kiær T, Ott P, Bondesen S & Tygstrup N. Serum Gc-globulin in the early course of multi-trauma. Critical Care Medicine 1998; 26(2): 285-289.

II. Dahl B, Schiødt FV, Nielsen M, Kiær T, Williams JG, Ott P. Admission level of Gc-globulin predicts outcome after multiple trauma. Injury 1999; 30: 275-281.

III. Dahl B, Schiødt FV, Ott P, Gvozdenovic R, Yin HL, Lee W. Plasma gelsolin is reduced in trauma patients. Shock 1999; 12: 102-104.

IV. Dahl B, Schiødt FV, Gehrchen PM, Ramlau J, Kiær T, Ott P. Gc-globulin is an acute phase reactant and an indicator of muscle injury after spinal surgery. Inflammation Research 2001; 50: 3 9-43.

V. Dahl B, Schiødt FV, Rudolph S, Ott P, Kiær T, Heslet L. Trauma stimulates the synthesis of Gc-globulin. Intensive Care Medicine 2001; 27: 394-399.

VI. Dahl B, Schiødt FV, Ott P, Wians F, Lee WM, Balko J, O'Keefe GE. Plasma concentration of Gc-globulin is associated with organ dysfunction and sepsis after injury. Critical Care Medicine 2003; 31: 152-156.

VII. Rothenbach PA, Dahl B, Schwartz JJ, O'Keefe GE, Yamamoto M, Lee WM, Horton JW, Yin HL, Turnage RH. Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. Journal of Applied Physiology 2004; 96: 25-31.

Abbreviations

AIS Abbreviated injury scale
ARDS Acute respiratory distress syndrome
EASS Extracellular actin scavenger system
Gc Group-specific component
ISS Injury severity score
MODS Multiple organ dysfunction syndrome
RIE Rocket immunoelectrophoresis
SIRS Systemic inflammatory response syndrome

Introduction

In spite of a vast number of studies over the last three decades, the pathophysiological processes taking place after severe trauma are not yet fully understood. New knowledge about these processes is of importance since post-trauma physiology is responsible for the potential development of trauma induced organ dysfunction. A number of theories have been proposed, reflecting the complexity of the condition. Originally thought to be caused by an occult intraabdominal infection initiating a cascade of organ symptoms often presenting with lung dysfunction, multiple organ dysfunction was later recognized as a condition not necessarily initiated by infection since a bacterial focus was not always identified (Border, 1992).

The idea that the initial inflammatory response is basically an appropriate physiological reaction was underlined by the definition of an inflammatory state named Systemic Inflammatory Response Syndrome (SIRS) (Bone, 1996). According to this hypothesis, patients sustaining a severe trauma are often resuscitated into a state of early SIRS which is probably of benefit to the patient and gradually normalizes if the patient recovers. If, however, a state of hyperinflammation develops SIRS can gradually evolve into Multiple Organ Dysfunction Syndrome (MODS), a condition associated with a significant mortality (Brun-Buisson, 2000).

Within the last ten years there has been increased focus on the role of cytokines in the development of MODS; primarily TNF-α, IL-1 and IL-6 (Dinarello, 1996; Nast-Kolb et al., 1997). Although not yet fully understood, the role of cytokines is supported by the finding that circulating levels of some cytokines are elevated in both animals and humans with severe SIRS and MODS. Also, injection of an inflammatory cytokine like IL-1 induces MODS and attempts have been made to block this effect of inflammatory cytokines using either cytokine antibodies or receptor blockers (Napolitano et al., 1999; Sherry et al., 1994).

Although the mortality of trauma induced MODS has decreased over the last decade, the morbidity remains high (Lee et al., 2001). Therefore, it is of relevance to obtain further knowledge about the physiologic reactions taking place after severe trauma. Such knowledge would increase the possibility of discovering new therapuetic modalities, and also increase the likelihood of identifying markers making it possible to select patients at risk for complications.

The possible role of the Extracellular Actin Scavenger System (EASS) in the development of organ dysfunction was initially described in patients suffering from fulminant hepatic failure (Schiødt et al., 1997). Since one of the proteins of the EASS, Gc-globulin, is primarily produced in the liver, it was hypothesised that liver dysfunction would result in decreased levels of circulation Gc-globulin. Also, Gc-globulin plays a role in clearing the plasma from intracellular actin released from dead liver cells. Therefore, it was speculated that an increased complex formation between Gc-globulin and actin would take place. This hypothesis was confirmed, and furthermore it was shown that the levels of Gc-globulin correlated with the degree of organ dysfunction.

Since the pathophysiology of MODS is thought to be similar regardless of the initiating insult, it could be speculated that the circulating levels of the two proteins of the EASS are also affected in trauma patients who often develop varying degrees of organ dysfunction.

Aim of the present studies

The detailed aims of the present thesis were:

1. To characterize the early changes in circulating levels of the proteins of the EASS after severe injury.
2. To compare changes in proteins of the EASS with changes in known acute phase reactants after major surgery
3. To evaluate whether admission plasma levels of the proteins of the EASS correlated with survival and the development of organ dysfunction after severe trauma.

4. To compare the changes of the EASS in trauma patients with changes seen in an experimental animal model of the inflammatory response after severe trauma.

These results could give important new information about the role of the EASS in severe trauma and major surgery. This knowledge could possibly result in new therapeutic and prognostic tools in the treatment of trauma patients.

The extracellular actin scavenger system (EASS)

The plasma proteins Gc-globulin and gelsolin represent the circulating elements of the EASS, being responsible for the continuous removal of actin from the circulation. Actin is the primary intracellular protein constituting up to 20% of total cellular protein, and it plays a crucial role as a part of the cytoskeleton. Also, its ability to polymerize and depolymerize characterizes actins role in cell motility (Safiejko-Mroczka and Bell, Jr., 2001). Apart from maintaining cell structure actin may play a role in the regulation of epithelial Na^+ channels (Cantiello et al., 1991; Undrovinas et al., 1995; Berdiev et al., 1996). Furthermore, actin may serve to store and release calcium, thereby being involved in the intracellular calcium signalling (Lange and Brandt, 1996).

Actin is released to the extracellular environment both as a result of normal cell turn-over and cell death, but also following more pronounced tissue death. The extracellular environment favours the formation of actin filaments (F-actin), consisting of monomeric globular actin units (G-actin). If these filaments are not continuously removed from the circulation, experimental studies suggest that they may be involved in the pathogenesis of Acute Respiratory Distress Syndrome (ARDS). By infusing increasing amounts of actin into rats, a clinical condition resembling ARDS developed, with intravascular filament formation, microthrombi and endothelial injury especially in the pulmonary circulation (Haddad et al., 1990).

Gc-globulin

Gc-globulin (Gc = group-specific component) is a 58-kd plasma-protein and though its initial physiological function was described as a carrier protein for vitamin D metabolites (DBP = Vitamin D binding protein) less than 5% of circulating Gc-globulin is complexed with vitamin D metabolites (Lee and Galbraith, 1992).

The gene coding for Gc-globulin is located on chromosome nr. 4 in close proximity to the gene coding for albumin, but as opposed to albumin, levels of Gc-globulin are not reduced in patients with cancer or inflammatory diseases (Rostenberg et al., 1979). Normally, circulating levels vary between 290 and 380 mg/L depending on the method of analysis, and increased levels are only seen in pregnant women in the third trimester. Like most plasma proteins, Gc-globulin is synthesized in the liver (Haddad et al., 1983). Using human hepatoma-derived cells lines it has been shown that approximately 3% of the protein synthesized by the cells had characteristics like Gc-globulin.

Several cell lines have been examined for a possible expression of the Gc-globulin gene including B- and T-lymphocytes, but any extrahepatic synthesis of Gc-globulin has not yet been demonstrated (Gouth et al., 1990).

The function of Gc-globulin in the EASS is to form complexes with monomeric actin (Figure 1). This, however, requires the participation of gelso-

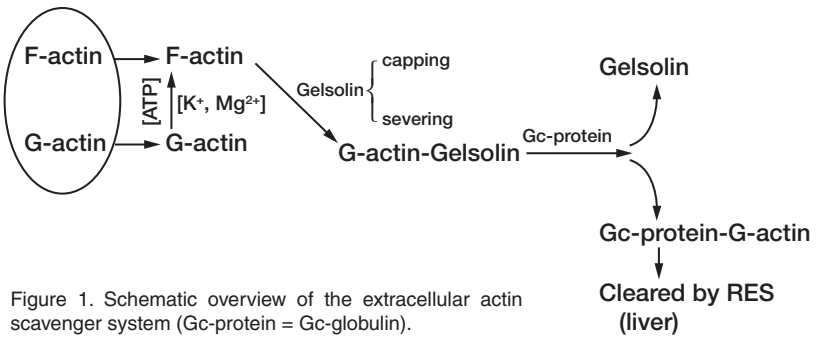

Figure 1. Schematic overview of the extracellular actin scavenger system (Gc-protein = Gc-globulin).

lin, the other EASS protein. When actin is released to the extracellular environment it polymerizes into actin filaments (F-actin). The role of gelsolin is to bind to the sides of the F-actin filaments. This process is known as "severing" and results in the breakage of the filaments in two. At the same time, gelsolin binds to the barbed ends of the severed F-actin filaments; a process termed "capping". The combination of these processes inhibits the addition of monomers to F-actin and also de-polymerizes F-actin. Subsequently, Gc-globulin binds to the monomeric actin (G-actin) and these complexes of Gc-globulin and actin are primarily cleared in the liver, and to a smaller extend in the spleen and the kidneys (Lee and Galbraith, 1992).

In serum from pregnant women an increased amount of Gc-globulin actin complexes has been found, possibly as a result of trophoblast material entering the maternal circulation (Emerson et al., 1983).

The plasma half life of Gc-globulin is 12 to 24 hours, but complex formation with actin markedly decreases the plasma half life to a magnitude of around 30 min. (Lind et al., 1986; Harper et al., 1987; Goldschmidt-Clermont et al., 1988).

Gelsolin

Gelsolin exists in a secretory and an intracellular form. The gene coding for both types of gelsolin is located on chromosome no. 9 (Kwiatkowski et al., 1988c; Kwiatkowski et al., 1986). Plasma gelsolin is considered to be a variant of cellular gelsolin, and the term "gelsolin" refers to it's ability to convert F-actin from a ***gel*** to a ***sol***vent state through a rapid shortening of actin filaments (Kwiatkowski et al., 1986; Young et al., 1987).

Together with a group of actin-binding proteins named villin, fragmin, and severin (Way et al., 1989), intracellular gelsolin plays an important role in maintaining cell shape through remodelling of the cytoskeleton and the regulation of movement and of the cytoplasm (Yin et al., 1981; Yin et al., 1980). As previously described, the role of secretory gelsolin in the EASS is to de-polymerize actin filaments into monomeric actin units. Gelsolin only binds to actin in the presence of Ca^{2+}. In the absence of Ca^{2+}, gelsolin consists of six structurally related domains, packed together in such a way that the actin binding sequences are not sufficiently exposed (Burtnick et al., 1997).

Skeletal muscle is the primary source of plasma gelsolin (Kwiatkowski et al., 1988a), although a gelsolin like protein is secreted by macrophages (Johnston et al., 1990; Kwiatkowski et al., 1988b; Yin et al., 1984). The half-life of gelsolin in plasma is 2.3 days, but no study so far has estimated the half-life for gelsolin bound to actin (Smith et al., 1987). It has been demonstrated that the rate of synthesis of gelsolin can be enhanced by dexamethasone treatment (Lanks and Kasambalides, 1983).

Gelsolin exists in a cytoplasmic and secreted form. Skeletal, cardiac, and smooth muscle have large amounts of plasma gelsolin mRNA, and an estimated 3% of these cells biosynthetic activity is devoted to synthesis of plasma gelsolin, however, due to its larger fraction of body mass, skeletal muscle is the major source of plasma gelsolin (Kwiatkowski et al., 1988a).

Methods of analysis

Three primary methods have been reported in the literature for measurements of circulating levels of Gc-globulin and gelsolin: rocket immunoelectrophoresis, nephelometry, and Western blotting

Rocket immunoelectrophoresis

Measurements of circulating levels of Gc-globulin have been done using rocket immunoelectrophoresis (RIE) (Goldschmidt-Clermont et al., 1985). Based on the complex formation between purified Gc-globulin and actin, increased rocket heights in an agarose gel varied in a log linear fashion with the amount of actin present in the sample. The advantage of this analysis is that the following Gc-globulin variables are obtained; concentration of total Gc-globulin and the percentage of total Gc-globulin bound to actin. Based on these parameters the concentration of Gc-globulin unbound to actin, so called free Gc-globulin, and the concentration of Gc-globulin bound to actin can be calculated.

Immunonephelometry

The principle of immunonephelometry is based on a positive linear relationship between the scattering

of a light source by anti-gen-antibody complexes and the concentration of the antigen (e.g. Gc-globulin). Using nephelometry it is possible to measure the circulating concentrations of total Gc-globulin and gelsolin (Suhler et al., 1997) (Wians et al., 1997). One advantage of this method is that it is less time consuming compared to rocket immunoelectrophoresis. Also, a nephelometer is already used in many clinical laboratories.

Western blotting

Western blotting has primarily been used for measuring gelsolin concentrations in both human and animal studies. After electrophoresis the blotting is performed with antibodies specific for gelsolin reacting specifically with the protein. This results in immunoreactive bands and the intensity of these bands can be determined by densitometry expressing the optical density per mm^2 of the stained band. In the present thesis the same principle was used for the measurement of plasma concentrations of Gc-globulin (Rothenbach et al., 2003).

Non-actin scavenger functions of the EASS proteins

Besides scavenging of actin and transport of vitamin D, accumulating evidence suggests that Gc-globulin also plays a role in the function of the immune system. Through the action of enzymes on B- and T-cells, Gc-globulin can be converted into a macrophage activating factor (Yamamoto and Kumashiro, 1993). A recent study into this aspect of Gc-globulin function indicated that Gc-globulin, once converted into a macrophage activating factor, inhibits angiogenesis through a direct effect on the endothelium and also stimulated macrophages to attack tumor cells (Kisker et al., 2003).

An interaction between gelsolin and macrophages has also been found since a gelsolin-like protein has been found in macrophages and also secreted from macrophages (Johnston et al., 1990). This finding probably reflects gelsolins role as a modulator of the cytoskeleton on macrophages, but a more complex function of the protein in myeloid cell differentiation is possible, since an increase in gelsolin expression is essential during the differentiation of myeloid cell lines into macrophages (Kwiatkowski, 1988).

While it is not possible to detect Gc-globulin in the membranes of resting T-lymphocytes, Gc-globulin can be demonstrated on activated T-lymphocytes in relation to the Fc-receptor (Petrini et al., 1985; Machii et al., 1986). Gc-globulin is also expressed on B-lymphocytes (Petrini et al., 1983) and in the membrane of circulating monocytes (McLeod et al., 1986), indicating a cell membrane role and a possible modulating effect of vitamin D on lymphocytes (McLeod et al., 1986). Also, Gc-globulin antiserum inhibits human natural killer cell activity (Chujo et al., 1989).

Several authors have reported an interaction between Gc-globulin and neutrophils. It has been demonstrated that neutrophils bind exogenous Gc-globulin generating a C5a co-chemotactic effect depending on a continuous supply of Gc-globulin (Binder et al., 1999; Robbins and Hamel, 1990; Kew et al., 1995).

A number of studies have described the endotoxin binding capacity of Gc-globulin. Initially it was demonstrated that Gc-globulin binds to endotoxin of Escherichia coli, and that rats injected with endotoxin had a significant reduction in circulating levels of Gc-globulin (Berger and Beger, 1987; Watt et al., 1989). A clinical role of these findings has been suggested in patients with peritonitis, since levels of Gc-globulin could predict organ failure in this condition (Berger et al., 1990).

Finally, Gc-globulin in combination with gelsolin, has been shown to inhibit actin-induced platelet aggregation (Vasconcellos and Lind, 1993).

All of these findings suggest that the proteins of the EASS exert a more complex physiological function than merely clearing the circulation for actin released from dead cells and tissue.

The EASS in the early phase after trauma

Until 1998 only few studies had described changes in the proteins of the EASS after major tissue injury. Miskulin assessed the changes in various acute phase reactants after burn injury (Miskulin et al., 1978). A number of acute phase reactants were studied and patients were followed for eight days. An increased concentration in the acute phase proteins orosomucoid and haptoglobin was observed, whereas this was not the case for Gc-globulin. It was concluded that the production of acute phase proteins was induced by the production of proteases from injured tissue stimulating the liver to production of acute phase proteins. This study, however, only measured protein levels several days after burn injury, and therefore did not assess any early changes.

For the next ten years there was limited interest in the EASS. The primary focus was on the role of the proteins in sepsis and liver failure. In a study by Lee (Lee et al., 1989) patients with sepsis were shown to have reduced levels of Gc-globulin compared to healthy controls. Also, the percentage of Gc-globulin bound to actin was increased and correlated with the prognosis. Since Gc-globulin is primarily synthesized in the liver, it seemed of relevance to study the changes of the protein in patients with varying degrees of liver failure. A number of clinical studies have demonstrated markedly reduced levels of both Gc-globulin and gelsolin in such patients. Also, independent studies have shown a correlation between prognosis and Gc-globulin levels, both regarding survival and the development of multiple organ dysfunction (Schiødt et al., 1997; Lee et al., 1995).

In 1974 the first study describing the changes of Gc-globulin in surgical trauma included ten men undergoing hernia surgery in general anaesthesia (Wandall, 1974). Serum concentrations of 17 proteins were measured and it was found that the circulating concentration of Gc-globulin was reduced to 82% of pre-operative levels three hours after induction of anaesthesia. Ten years later a study on patients with hip fractures showed that circulating levels of Gc-globulin were marginally lower than in control subjects (Lips et al., 1985). Also, the levels correlated positively with both serum total protein and albumin (Lips et al., 1985). In the first study specifically aimed at studying early changes in circulating levels of Gc-globulin in trauma patients twelve consecutive patients with an Injury Severity Score (ISS) > 15 were included (Dahl et al., 1998). The injury severity score (ISS) was introduced in 1974 (Baker et al., 1974) and has gained wide acceptance as an anatomical scoring system. It is based on the Abbreviated Injury Scale (AIS) 1990 Revision (Association for the Advancement of Automotive Medicine, 1990). Each injury is categorized by severity with one point for minor injury and six points for critical lesions. The ISS is obtained by summing the squares of the three highest AIS scores in five body regions. Most trauma studies defines severe injury as patients having an ISS > 15, corresponding to a mortality of ≥ 10%.

All patients had a blood sample taken on admission. The concentration of Gc-globulin and complex formation between Gc-globulin and actin was measured using rocket immunoelectrophorese (Goldschmidt-Clermont et al., 1985).

The results showed that admission levels of Gc-globulin was significantly reduced compared to healthy controls, combined with a significant increase in the complex formation between Gc-globulin and actin. The latter finding confirmed that the reduced level of Gc-globulin was the result of a specific load on the EASS and not merely a dilution phenomenon. This was further supported by the fact that none of the patients had received intravenous fluid prior to the first blood sample. No difference in admission values was found between survivors and non-survivors, but the mean level the first week after the trauma correlated with the ISS, indicating that admission levels of Gc-globulin possibly correlated with the outcome

This first study describing the EASS in trauma patients had a number of limitations. Besides the relatively small number of patients some of the blood samples were taken several hours after the trauma. This made it difficult to assess the role of

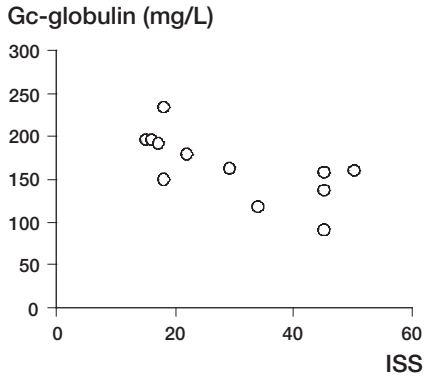

Figure 2. Correlation between ISS and mean Gc-globulin the first week after trauma ($r = -0.72$, $p < 0.05$, Spearman's test) (Dahl et al., 1998).

of the EASS regarding survival and development of complications.

Until 1999 no study had focused on the role of gelsolin in the pathophysiology of severe trauma. The first study on this aspect of the EASS included 23 consecutive trauma patients with an ISS > 15 (Dahl et al., 1999b). In all patients a blood sample was obtained within two hours after the injury, and in a subset of eleven patients, blood samples were obtained the first week of hospitalization. Gelsolin levels were measured in serum using immononephelometry. Although the gelsolin levels were significantly reduced compared with normal controls, there was no correlation between admission levels of gelsolin and severity of the injury or survival. In patients with serial blood samples the lowest levels of gelsolin were found on day one, with a subsequent gradual increase towards normal values over the first week. Again, no correlation with injury severity or survival could be demonstrated.

These initial studies supported the theory that the EASS is involved in the pathophysiological processes taking place after severe injury. The studies, however, also indicated differences in the role of the two proteins constituting the actin scavenger system. One explanation for the lack of correlation between gelsolin levels and degree of injury and survival, could be the fact that gelsolin is primarily synthesized in skeletal muscle. Since most trauma, to a varying degree, is accompanied by muscle injury any drop in circulating levels of gelsolin could be counterbalanced by the release of gelsolin from injured muscle tissue. The finding that admission levels of gelsolin was reduced in trauma patients was supported in a study focusing on the role of gelsolin and the development of ARDS (Mounzer et al., 1999). That study focused specifically on ARDS and it was found that circulating levels of gelsolin below 250 mg/L were associated with prolonged mechanical ventilation. One mechanism explaining the correlation between pulmonary problems and gelsolin could be a toxic role of actin on the pulmonary tissue as previously described in experimental studies infusing increasing amounts of actin into rats (Haddad et al., 1990). By infusing increasing amounts of actin into rats a clinical condition resembling ARDS developed with intravascular filament. A study focusing on the toxic role of actin confirmed this hypothesis (Erukhimov et al., 2000). It was shown that sera from patients with ARDS were toxic to cultured sheep pulmonary endothelial cells. The same result was obtained using normal serum supplemented with actin. Preincubation of both types of sera with gelsolin inhibited this effect.

Considering the relatively long half life of Gc-globulin around 24 hours, it seems remarkable that serum concentrations could decrease to half the level of normal values in such a short time after injury. One explanation could be that the half life of Gc-globulin is markedly reduced to around 30 min, when Gc-globulin is complexed with actin. In the initial study the authors estimated that the reduction in free Gc-globulin was larger than could be explained by an increase in Gc-globulin complexed to actin (Dahl et al., 1998). They therefore suggested that mechanisms other than actin clearance could explain the early reduction in Gc-globulin after injury.

All of these studies confirmed the hypothesis that the proteins of the EASS play a role in the pathophysiological processes taking place after severe injury.

Gc-globulin as an acute phase reactant

Gc-globulin compared to known acute phase reactants

Although Gc-globulin is a liver synthesized plasma protein it has not generally been considered an acute phase reactant. Serial measurements of Gc-globulin in trauma patients, however, revealed an increase in circulating levels; in some cases resulting in levels higher than healthy controls one week after injury (Dahl et al., 2001b). This study showed that circulating levels of Gc-globulin decreased to levels as low as 50% of normal values on day one after injury, thereafter increasing to supranormal levels on day 7. It could be argued that this increase was a result of a decreased load on the actin scavenger function of the protein, resulting in a "passive" increase in plasmalevels of Gc-globulin. Combined with the fact that levels of Gc-globulin complexed with actin, normalized after three days, this was an indication that Gc-globulin displays features similar to known acute phase reactant. The serial measurements of Gc-globulin also compared levels of Gc-globulin the first week after trauma in survivors and non-survivors. It was found, that after a nadir on day one for both groups the levels of both total Gc-globulin and free Gc-globulin was significantly lower in non-survivors compared to survivors. This study supported the role of Gc-globulin in the prognosis after trauma, but also shed light on the dynamic aspects of the protein. The serial measurements of Gc-globulin resembled that of an acute phase response. This is supported by the fact that the cytokine interleukin-6 (IL-6) increases the amount of mRNA coding for Gc-globulin in hepatocytes (Guha et al., 1995). It could therefore be speculated that the increase in Gc-globulin levels after the initial reduction is the result of IL-6 stimulated synthesis in the liver.

For obvious reasons pre-injury levels of Gc-globulin was not available in trauma patients. It was therefore of relevance to design a study on patients undergoing an elective surgical procedure, inducing a pathophysiological condition with parallels to a trauma situation. This would allow determination of Gc-globulin levels prior to a surgical trauma. A series of patients undergoing posterior lumbar spinal fusion was chosen, since this type of surgery is associated with a muscular trauma releasing actin to the circulation, resulting in a load on the EASS. The magnitude of muscle injury after this type of surgery had previously been studied (Kawaguchi et al., 1997).

In twelve patients undergoing one or two level lower lumbar fusion, pre-operative levels of Gc-globulin, albumin, orosomucoid, transferrin, and haptoglobin were measured (Dahl et al., 2001a). To verify the magnitude of muscular trauma, levels of creatin phosphokinase were also measured. The study confirmed that circulating levels of Gc-globulin display the characteristics of an acute phase reactant with an initial reduction on day one, followed by an increase over the first week, with levels still 30% above pre-surgical levels four weeks after surgery (Figure 3).

As previously described, Gc-globulin and albumin belong to the same protein family with their encoding genes closely located on chromosome no. 4. This is of relevance considering the fact that the plasma concentration of the two proteins differed with levels of albumin remaining low showing no phase reactant pattern. This suggests that the synthesis of Gc-globulin and albumin are regulated differently in the post-trauma inflammatory response, supporting the in vitro studies showing that IL-6 upregulates mRNA coding for Gc-globulin in hepatocytes whereas IL-6 and IL-1 decreases the sythesis of albumin by hepatocytes (Castell et al., 1990; Mackiewicz et al., 1991; Guha et al., 1995).

A similar pattern was not demonstrated for gelsolin in trauma or patients with acute liver failure (Dahl et al., 1999b; Suhler et al., 1997).

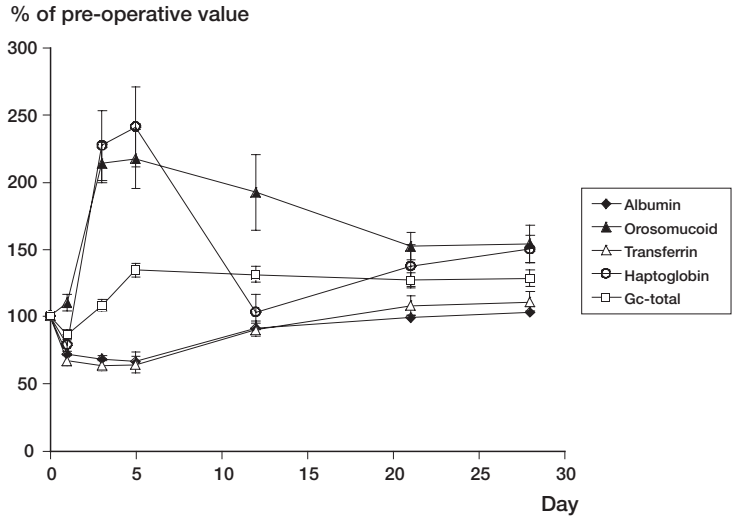

Figure 3. Acute phase reactants and Gc-globulin in 12 patients undergoing spinal fusion (Dahl et al., 2001a)

The EASS and prognosis after severe injury

Research into the early pathophysiological processes taking place in the inflammatory response after severe trauma is of relevance since it may reveal markers that can identify patients at increased risk of developing complications. The consequence of the inflammatory reactions taking place after severe injury has been termed the Systemic Inflammatory Response Syndrome (SIRS) (Muckart and Bhagwanjee, 1997). Cytokines are increasingly being recognised as a key factor in the development of SIRS; a condition sometimes developing into Multipel Organ Dysfunction Syndrome (MODS). One of the primary inflammatory mediators is interleukin-6 (IL-6) produced by a number of cells including T-cells and monocytes (Biffl et al., 1996). Other so called inflammatory cytokines include tumor necrosis factor β (TNF-β) and interleukin-1 (IL-1) (Bone, 1996). There are numerous studies supporting that TNF-β may be responsible for the hemodynamic changes seen in SIRS/MODS (Stephens et al., 1988). Based on these findings it is believed that TNF is released into the circulation immediately after injury resulting in endothelial cell injury and leakage of plasma macromolecules, leading to a loss in intravascular volume. This could explain the clinical finding of hypotension and tachycardia in SIRS, and also indicate a mechanism whereby an increase in microvascular permeability leads to edema of several organs. One theory regarding the role of TNF in the development of MODS focuses on the interaction between the microvascular endothelium and leukocytes. It has been shown that TNF has a direct effect on both neutrophils and endothelial cells resulting in an increased adherence between neutrophils and endothelial cells leading to endothelial injury (Varani et al., 1988). This injury may result in migration of neutrophils into the interstitium (Remick et al., 1987). It has been demonstrated that TNF can induce pulmonary edema, possibly through this neutrophil-dependent mechanisms (Lo et al., 1992). The adhesion is thought to require interaction between CD18 integrins on neutrophils and intracellular adhesion molecules (ICAM-1) on endothelial cells.

The release of inflammatory cytokine is accompanied by the release of anti-inflammatory cytokines like interleukin-10 (IL-10) attenuating the migration of neutrophils and diminishing further release of IL-6. and other inflammatory reactions (Neidhardt et al., 1997). Several studies have investigated changes in circulating levels of interleukins and their possible correlation with development of organ dysfunction and survival. In one study including 31 patients with ISS ranging from 9 to 57 a correlation between increased levels of IL-6 and ISS was found (Giannoudis et al., 1998). Admission levels of IL-6, however, were not usefull for the diagnosis of sepsis. There have been conflicting reports on the clinical use of cytokines in the identification of patients at risk of developing post-trauma organ dysfunction. In one study, plasma samples were obtained from severely injured patients with ISS ≥ 25 within one hour after the injury (Donnelly et al., 1994). Samples were repeated at four hours intervals and analyzed for TNF-α, IL-1β, IL-6, and IL-8. In spite of these short time intervals levels of these cytokines were not able to predict or identify patients with ARDS any sooner than clinical signs of pulmonary dysfunction were evident. In another study serum levels of TNF-α were significantly higher in patients with ARDS compared to normal control, whereas this was not the case for IL-1β and IL-6 (Bauer et al., 2000). In that study, however, samples were obtained within 24 hours of the onset of ARDS. This reflects that the timing of blood sampling for cytokine analysis is crucial, possibly due to the short half-life of most cytokines.

All of these mediators and pathophysiological mechanisms are linked to the risk of developing multiple organ dysfunction after trauma. The research in the different causes of Multiple Organ Dysfunction Syndrome (MODS) indicates that the pathophysiological processes are similar, independent of the initiating insult. In most cases, MODS is the result of progressive physiologic dysfunction in organ systems remote from the site of the primary disease process (e.g. infection, intaabdominal abcess, mul-

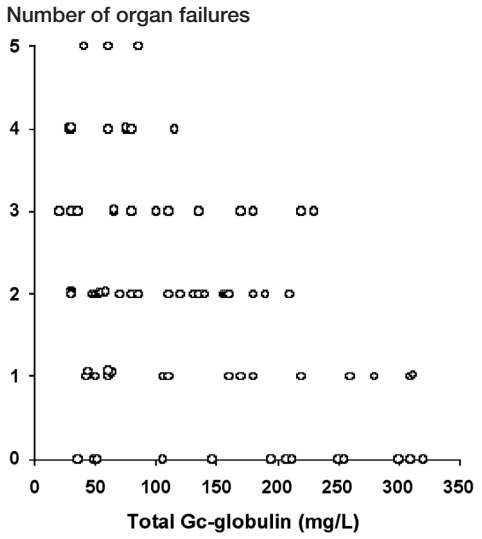

Figure 4. The correlation between admission levels of Gc-globulin and the number of organ failures in patients with fulminant hepatic failure (Schiødt et al., 1997). Reproduced with permission.

tiple fractures), possibly as a result of hyperinflammation shortly after the initiating insult. Although the clinical presentation of MODS after different insults appears to be similar, trauma patients represent the advantage in the study of MODS that the time of the insult can be estimated with more precision compared to conditions like gram-negative sepsis and intraabdominal infections.

The first detalied observations regarding a correlation between circulating levels of Gc-globulin and organ dysfunction was reported in patients with fulminant hepatic failure (Schiødt et al., 1997). Including 97 patients with hepatic encephalomathy it was shown that levels of total Gc-globulin and free Gc-globulin were significantly reduced compared to normal controls. Also, admission levels of Gc-globulin correlated with the number of organs that subsequently failed (Figure 4).

Looking specifically at the individual organ systems it was found that levels of Gc-globulin were significantly lower in patients who developed cardiovascular failure, intracranial hypertension, and infection but not in patients who developed renal or pulmonary failure. The study also supported previous findings that the predictive values of serum concentrations of Gc-globulin on admission were comparable to that of more advanced scoring systems (Lee et al., 1995).

Patients with hepatic failure were also included in the first published results regarding levels of gelsolin in severe disease (Suhler et al., 1997). That study, however, did not systematically investigate organ dysfunction but a correlation between gelsolin levels and severity of illness was found.

The initial studies of Gc-globulin and gelsolin established the fact that circulating levels of both proteins are reduced compared to normal levels shortly after severe trauma (Dahl et al., 1998; Dahl et al., 1999b). In these studies, however, some of the blood samples were obtained several hours after the injury, and the number of patients was small. Although the study on gelsolin in trauma patients (Dahl et al., 1999b) failed to demonstrate any relationship between concentration on admission and survival, a relationship between admission levels of gelsolin and development of ARDS in traumapatients has been demonstrated (Mounzer et al., 1999). In that study blood sampling was done on 13 patients within four hours after a trauma resulting in an AIS of ≥ 3 for at least one anatomical region. There was a median ISS of 20 (range 9–43) but it was only possible to calculate the ISS in 11 of the patients. Healthy controls had gelsolin levels of 517 mg/L and the patients had 261 mg/L which was significantly lower. A level on admission of less than 250 mg/L significantly increased the risk of ARDS. This was partly in contrast to other findings where no relationship was found between gelsolin levels and survival or ISS, although decreased levels was found compared to healthy controls (Dahl et al., 1999b). This difference could be explained by differences in types of trauma or timing of the blood sampling. As previously discussed the fact that gelsolin is primarily synthezised in skeletal muscle could mean that changes in circulating levels of the protein is counterbalanced by the simultaneous release from injured muscle tissue. This could also explain the different findings regarding gelsolin levels and prognosis after trauma.

Prognosis after severe injury can be assessed in several ways. One method is the so called TRISS-Like score using systolic blood pressure, age and best motor response on admission to calculate the probability of survival (Offner et al., 1992). Using TRISS-Like as a point of reference the predictive ability regarding survival of admission levels of

Table 1. Comparison of predictive abilities of TRISS-Like and Gc-globulin regarding death with corresponding 95% confidence intervals (Dahl et al., 1999a)

	TRISS-Like (%)	Gc-globulin (%)
Sensitivity	56 (30–80)	56 (30–80)
Specificity	93 (80–98)	90 (77–97)
Positive predictive value	75 (43–95)	69 (39–91)
Negative predictive value	84 (71–94)	84 (70–93)

Gc-globulin has been reported in 57 patients with ISS ≥ 15 (Dahl et al., 1999a). Predictive values of Gc-globulin regarding survival were comparable to that of the TRISS-like method (Table 1).

Seen from a pathophysiological point of view this supports the previous results suggesting that early changes in circulating levels of Gc-globulin play an important role in the prognosis after severe injury. The clinical implication of this finding is the possibility of using Gc-globulin as a marker of severity of injury. This could either be done by combining Gc-globulin and known risk-factors or using Gc-globulin alone. The latter option would make it easier to assess prognosis than using the ISS, which require the patient to be fully diagnosed regarding detailed anatomical location of the injuries.

A significant number of patients develop post-trauma organ dysfunction. Combined with the fact that circulating levels of Gc-globulin remains depressed the first week after injury (Dahl et al., 2001b), it is of relevance to establish a possible relationship between levels of Gc-globulin and organ dysfunction.

Impaired function of one or several organs can be regarded as the final complication of an acute critical illness. Over the past 25 years, the terminology in this area has been inconsistent, making comparison of research results difficult. The condition was initially described as so called "sequential system failure" (Tilney et al., 1973). This complication was observed following surgery for ruptured abdominal aortic aneurysms. In 1975 a report described three patients who died of "multiple, progressive or sequential systems failure" (Baue, 1975). Through the 1980s the term Multiple Organ Failure (MOF) and Multiple System Organ Failure (MSOF) (Fry et al., 1980) dominated the literature. One of the problems with the dichotomous term "failure" is that it does not fully describe the dynamic nature of the condition.

Multiple Organ Dysfunction Syndrome (MODS) was defined in 1992 by The American College of Chest Physicians and the Society of Critical Care Medicine as the "presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention" (Members of the American College of Chest Physicians/ Society of Critical Care Medicine Consensus Conference Committee., 1992).

The initial clinical studies on the proteins of the EASS established the fact the there is an association between circulating levels of Gc-globulin and death after trauma (Dahl et al., 1999a;Dahl et al., 2001b). In a cohorte of 98 trauma patients the possible relationship between organ dysfunction and circulating levels of Gc-globulin was investigated (Dahl et al., 2003). The study confirmed previous studies demonstrating a relationship between survival and admission levels of Gc-globulin, and furthermore showed that patients who develop severe MOD or sepsis after trauma have a lower circulating plasma Gc-globulin concentration on admission than patients who do not develop these complications. Assessment of serial changes in plasma Gc-globulin showed that the levels were significantly reduced in patients who developed respiratory failure and sepsis.

Looking at the individual organ systems the highest correlation with admission levels of Gc-globulin was found for respiratory and hematological failure. The correlation with pulmonary dysfunction supported the previously reported experimental results, showing that serum from patients with ARDS contains actin, and exerts a toxic effect on pulmonary endothelial cells (Erukhimov et al., 2000). It also supported clinical observations in non-trauma patients populations with ARDS, thus supporting the accepted hypothesis that there is a common pathophysiological pathway for ARDS independent of the initiating insult (Emmett et al., 1987).

Compared with the situation in patients with fulminant hepatic failure, however, there was a difference since in these patients the highest correlation was found between Gc-globulin concentrations and cardiovascular failure, intracranial hypertension,

Table 2. Relationship between Gc-globulin concentration on admission and individual organ system failures. No patients were classified as having severe renal dysfunction and/or failure. Comparisons were made using the Pearson's chi-square, and actual p values are indicated

Organ system failure	Gc-globulin concentration ≤134 mg/L (%) (n=24)	>134 mg/L (%) (n=74)	*p* value
Hematologic	3 (13)	0 (0)	0.002
Respiratory	8 (33)	7 (9)	0.005
Cardiovascular	4 (17)	4 (5)	0.082
Hepatic	2 (8)	2 (3)	0.228
Renal	–	–	

and infections (Schiødt et al., 1997). One explanation for this difference could be that the pathophysiology, with regard to the EASS, in patients with hepatic failure is different than in patients with severe trauma. Hence, hepatic failure besides release of actin from necrotic hepatic tissue, results in direct affection of Gc-globulin synthesis since the protein is synthesized in the liver. This situation is uncommon in trauma patients where hepatic failure is relatively rare (Dahl et al., 2003).

The correlation between admission levels of Gc-globulin and hematological failure was assessed by measuring the degree of thrombocytopenia. Experimental studies have shown that actin filaments are able to aggregate platelets, possibly explaining the formation of microthrombi after injection of large amounts of actin into rats (Janmey et al., 1992; Janmey et al., 1985; Haddad et al., 1990). The combined effect of Gc-globulin and gelsolin is able to inhibit this aggregation and this could explain the correlation between thrombocytopenia and levels of Gc-globulin.

Results from animal experiments

The first clinical study focusing on Gc-globulin was based on the hypothesis that since Gc-globulin is synthesized in the liver, a clinical condition with hepatocyte necrosis will result in reduced levels of circulating Gc-globulin (Lee et al., 1985). This was confirmed in patients with acute hepatitis and the results indicated that the proportion of Gc-globulin complexed with actin was greater in patients with fulminant hepatic necrosis. Based on these clinical observations initial experimental studies on Gc-globulin was done in a hamster model of fulminant hepatic necrosis. These experiments confirmed the clinical observations showing reduced levels of Gc-globulin combined with increased formation of Gc-globulin-actin complexes (Lee et al., 1987). Also, a correlation between the extent of acetaminophen-induced the liver damage and degree of complex formation between Gc-globulin and actin was seen (Young et al., 1987).

The idea of a possible role for the proteins of the EASS and the development of lung injury first emerged as a result of observations in patients with ARDS (Lind et al., 1988). It was speculated that long actin filaments would result in an increase in plasma viscosity affecting the microvasculature, and this was confirmed by demonstrating reduced levels of gelsolin in 25 out of 25 patients with ARDS. In the majority of patients actin was detectable in the plasma as opposed to normal individuals. Using intravenously injection of oleic acid in rats thereby inducing acute hemorrhagic necrosis it was demonstrated that a reduction of circulation gelsolin took place. Based on the fact that the concentration of free gelsolin was reduced more than the total gelsolin concentration in was concluded that there was a presence of circulating actin-gelsolin complexes, indicating a specific load on the EASS (Smith et al., 1988).

A direct toxic effect of actin on the lungs has been demonstrated by intravenous injection of increasing amounts of actin into rats (Haddad et al., 1990). This resulted in intravascular actin filament formation, microthrombi, and endothelial injury and the effect could be abolished by pre-incubating the actin containing infusate with Gc-globulin.

Combined with the recent clinical studies linking circulating Gc-globulin and gelsolin to the development of pulmonary dysfunction in trauma patients further experimental efforts have been aimed in this direction. Pulmonary involvement in trauma patients is proceeded by a physiological condition termed systemic inflammatory response syndrome (SIRS) and the hypermetabolic state of SIRS can gradually develop into multiple organ dysfunction syndrome (MODS) (Bone et al., 1992). Regardless of the initiating insult being trauma, infection, or sepsis the evidence suggests that a common underlying pathway is responsible for the pathophysiology of SIRS. The possible mechanisms include complement activation, cytokines, and the activation of neutrophils displaying adhesion molecules resulting in vascular injury (Mulligan et al., 1994; Chen and Christou, 1998).

A number of experimental models have been developed to study the systemic inflammatory response (SIRS) after severe trauma. Pulmonary involvement is an invariable component of SIRS, regardless of the initiating insult, and the clinical study on organ dysfunction and plasma levels of Gc-globulin in trauma patients, confirmed that a high correlation between admission levels of Gc-globulin and respiratory failure exists (Dahl et al., 2003). Therefore, an experimental animal model inducing a pulmonary injury could contribute to the pathophysiological understanding of the role of Gc-globulin and gelsolin in severe trauma.

A standardized rat burn model has been widely accepted as way to induce a well defined pulmonary injury sharing the characteristics of pulmonary pathology following severe SIRS (Lightfoot E Jr et al., 1999; Mulligan et al., 1994; Walker and Mason, 1968). This model has been used in the only study on the proteins of the EASS under standardized conditions (Rothenbach et al., 2003). A rat burn model was used to induce a pulmonary injury applying a 40% total body surface area burn. The pulmonary injury was assessed by measur-

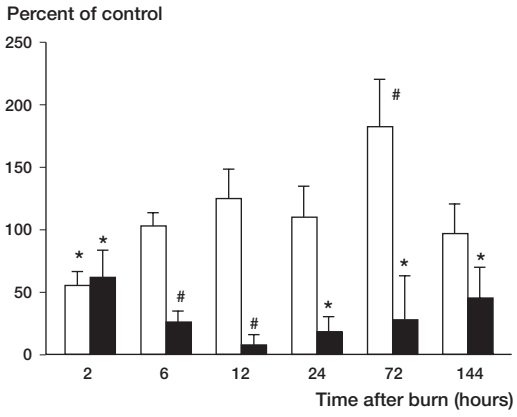

Figure 5. Plasma levels of gelsolin and Gc-globulin following a 40% total body surface burn in rats (Rothenbach et al., 2003). The bars represent levels of Gc-globulin (white bars) and levels of gelsolin (black bars) as a percentage of control values. Significant differences ($P < 0.05$) are indicated by # and *.

ing the pulmonary microvascular permeability in an ex vivo lung perfusion model. Following burn injury, plasma levels of Gc-globulin and gelsolin were measured at 2, 6, 12, 24, 72 and 144 hours. The experimental results confirmed the previously reported clinical observations in trauma patients (Dahl et al., 1999b;Dahl et al., 2001b). The plasma concentrations of both proteins were initially reduced; followed by an increase in Gc-globulin to supra-normal levels, while the circulating concentrations of gelsolin remained lower than control values throughout the observation period (Figure 5).

In burn injury it is known that plasma proteins are lost through the burn wound, and it is also known that plasma proteins are lost because of the increase in pulmonary vascular permeability. Therefore it could be argued that the reduction in circulating levels of gelsolin is caused by a general expansion of the intravascular fluid compartment by the infusion of crystalloid resuscitation fluids and as such a consequence of hemodilution. The finding that levels of gelsolin remained reduced when normalized to plasma protein levels, proves that hemodilution did not take place. Furthermore, the hematocrit of burn-injured animals at no point was less that that of the SHAM group. Although a part of the reduction in levels of gelsolin could be explained by hemodilution, the absolute reduction of circulating levels of gelsolin could cause a loss of its protective effect against the effects of inflammation on lung tissue.

The aspect of protein loss through the burn wound and through the pulmonary endothelium is only one important limitation of using this experimental setup, comparing the results with the clinical observation of changes in circulating levels of Gc-globulin and gelsolin in trauma patients. It could be argued that an experimental model including some kind of crush injury, would be a more precise reflection of the clinical conditions under which plasma levels of the two proteins have been measured. As previously mentioned, the primary reason for choosing the burn model was the ability of the model to assess the degree of pulmonary dysfunction, since the clinical results indicated a close correlation between lung dysfunction and admission levels of Gc-globulin. Also, other groups reported a correlation between plasma levels of gelsolin and development of ARDS. Combined with the possibility of injecting gelsolin to ameliorate the pulmonary injury the use of a burn injury model can be justified.

The pulmonary injury in the animals was assessed using an ex vivo lung perfusion mode. This made it possible to quantitate the microvascular permeability and pulmonary hemodynamics. It was found that burn injury induced an increased pulmonary microvascular permeability. This was reflected in the capillary filtration coefficient. However, it was possible to prevent this by infusion of recombinant human gelsolin prior to inflicting the burn wound (Figure 6).

This finding supports the clinically based studies suggesting that gelsolin plays an important role in protecting the lungs from inflammatory injury caused by SIRS. It is thus possible that circulating gelsolin protects the organism from lung injury which almost consistently is a part of the pathophysiology of SIRS.

In a recent study pulmonary vascular permeability following pulmonary ischemic injury was assessed in gelsolin-deficient mice (Becker et al., 2003). The study concluded that gelsolin-deficiency leads to increased lung permeability at baseline, but that this increase in lung permeability was even more pronounced following pulmonary ischemia. Although the consequences of gelsolin-deficiency primarily have been described

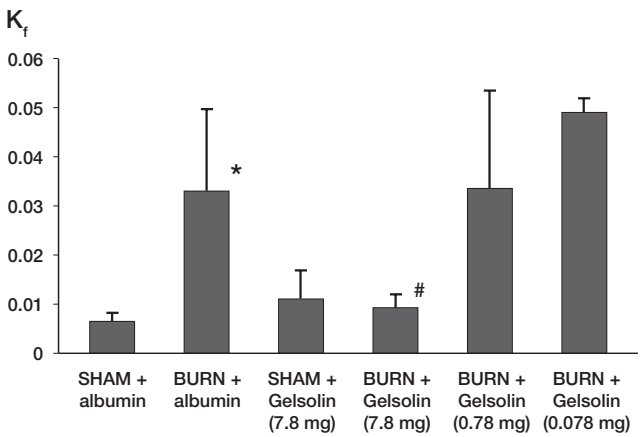

Figure 6. Capillary filtration coefficient Kf of lungs after burn injury with increasing concentrations of recombinant gelsolin infusion (Rothenbach et al., 2003).

as delayed neutrophil migration and prolonged bleeding time, the most likely explanation for the results in that study is that the gelsolin deficiency affected the cytoskeleton resulting in a dysfunction of the pulmonary vascular barrier. It could, however, be speculated the absence of plasma gelsolin resulted in a decrease in actin scavenging function leading to actin toxicity of the pulmonary endothelial cells. This is supported by experimental findings that actin-containing sera from patients with ARDS are toxic to sheep pulmonary endothelial cells (Erukhimov et al., 2000). A Gc-globulin deficient mouse line has also been developed (White et al., 2002). The primary goal of that study was to test the hypothesis that Gc-globulin plays a pivotal role in the immune response. This could not be confirmed. Unpublished studies show that it is possible to purify large amounts of Gc-globulin. This would be of relevance in future studies focusing on the consequences of infusion of Gc-globulin in experimental models of SIRS.

Conclusions and futures studies

The present review has addressed the aims defined earlier.

Clinical studies have characterized the early changes in plasma concentrations of Gc-globulin and gelsolin after severe injury:

1. The circulating levels of both proteins are significantly reduced shortly after trauma compared to normal controls. Furthermore the complex formation between Gc-globulin and actin is increased indicating a specific load on the Extracellular Actin Scavenger System.
2. The changes of Gc-globulin resemble that of an acute phase reactant, with an initial decrease followed by supra-normal levels the first week after injury and major surgery.
3. Admission plasma levels of Gc-globulin correlates with the subsequent development of organ dysfunction and survival.
4. The plasma levels of Gc-globulin and gelsolin in an animal burn injury model, resembles those seen after severe injury.

In conclusion, significant new knowledge about the proteins of the EASS in the pathophysiological processes taking place after severe trauma, has been established. Initially, changes in circulating levels of Gc-globulin and gelsolin have been described in patients with fulminant hepatic failure, but within the last five years a number of studies have documented that early changes in the concentration of the proteins are predictive of prognosis after severe injury, both regarding survival and development of organ dysfunction.

Experimental studies have supported the clinical findings, and indicated the possibility of a therapeutic use of gelsolin to attenuate the degree of pulmonary injury, but further experimental studies are needed to produce more detailed information about the kinetics of the two proteins after severe injury. In the near future experimental studies with infusion of Gc-globulin are realistic.

Summary in Danish

Det Extracellulære Actine Scavenger System (EASS) er ansvarlig for fjernelse af actin fra cirkulationen. Aktin er det dominerende intracellulære protein og frigives til ekstracellulærmiljøet i forbindelse med almindelig celle og vævs-turnover og i forbindelse med større vævsdød. EASS består af de to plasmaproteiner Gc-globulin og gelsolin. Gelsolin syntetiseres primært i skeletmuskulatur og er ansvarlig for nedbrydningen af actin filamenter til monomere actin enheder. Disse actinenheder danner komplekser med det andet protein i EASS, Gc-globulin der primært syntetiseres i leveren. Komplekserne cleares herefter i leveren.

Afhandlingen tilfører ny viden om den rolle som plasmaproteinerne i EASS spiller for de patofysiologiske forhold efter alvorlig tilskadekomst; både i forhold til overlevelse men også i forhold til risikoen for udvikling af organkomplikationer. Hos traumepatienter er plasmakoncentrationen af både Gc-globulin og gelsolin reduceret i forhold til normale kontrolpersoner. Denne reduktion ses allerede inden for få timer efter tilskadekomsten, og plasmakoncentrationen af Gc-globulin ved ankomsten til skadestuen er prædiktiv for overlevelse; hvorimod dette ikke synes at være tilfældet når det gælder plasmakoncentrationen af gelsolin. I den første uge efter såvel svær tilskadekomst som større kirurgi øges plasmakoncentrationen af Gc-globulin til værdier over normalområdet, og dette indikerer at Gc-globulin er en akutfase reaktant. Dette er bekræftet i undersøgelser af ændringerne i Gc-globulin sammenlignet med kendte akutfase reaktanter hos rygopererede patienter

Den prognostiske værdi af Gc-globulin for overlevelse er sammenlignelig med traditionelt anvendte traumescoringssystemer, der erfaringsmæssigt tager længere tid at udføre og som kan være forbundet med nogen usikkerhed bl.a. fordi nogle elementer i disse scoringssystmer er vanskelige at belyse hvis patienten eksempelvis er intuberet. Denne sammenhæng mellem overlevelse og plasmakoncentration er ikke helt så entydig for gelsolin, og skyldes muligvis at gelsolin syntetiseres i skeletmuskulatur. Ved tilskadekomst er det således muligt at den cirkulerende mængde gelsolin er en sum af den eksisterende plasma gelsolin, samt gelsolin frigjort fra ødelagt musklulatur.

Ud over sammenhængen mellem Gc-globulin koncentrationen og overlevelse påvises også en sammenhæng med risikoen for udvikling af organ dysfunktion.

De observerede ændringer hos traumepatienter er bekræftet i et eksperimentelt studium hvor brandsårsskader hos rotter resulterede i de samme ændringer af de to EASS proteiner som man ser hos traumepatienter. Endvidere påvistes det at infusion af gelsolin yder en beskyttende effekt på den lungeskade der ses efter brandsårsskaden.

Det konkluderes at der er opnået betydelig ny viden om rollen af Gc-globulin og gelsolin i det patofysiologiske forløb efter svær tilskadekomst. Den kliniske betydning af denne viden kan bedst vurderes hvis der i fremtiden udvikles analysemetoder der er egnede til rutinebestemmelse af de to proteiner.

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