*Clinical Chemistry* 52:7
1247–1253 (2006)

Reviews

# Gc-Globulin: Roles in Response to Injury

Ursula Meier,[1*] Olav Gressner,[2] Frank Lammert,[2] and Axel M. Gressner[1]

***Background:*** **Gc-globulin (vitamin D–binding protein) appears to have important functions in addition to its role as a carrier of vitamin D.**

***Approach:*** **We reviewed recent studies focusing on the pathophysiologic functions and clinical significance of Gc-globulin.**

***Results:*** **Serum concentrations of Gc-globulin, as determined by immunoassay techniques, are decreased in severe injury. The extent of the decrease may have prognostic significance for patient outcomes. Clinical studies and animal models have shown that Gc-globulin has an important role in the clearance of procoagulant actin from the circulation after its release during cell necrosis and tissue injury. Gc-globulin has other potential roles in responses to acute tissue injury through conversion to a macrophage-activating factor, neutrophil chemotactic activity, and enhancement of C5a-mediated signaling.**

***Conclusion:*** **Considering the important physiologic roles of Gc-globulin in responses to tissue injury, such as clearance of actin, measurement of Gc-globulin may have value in directing the care of patients in many clinical disorders.**

**© 2006 American Association for Clinical Chemistry**

Group-specific component globulin (Gc-globulin),[3] also known as vitamin D–binding protein, is a multifunctional plasma protein with a relative molecular mass ($M_r$) of 51 000–58 000 and belongs to the albumin superfamily of binding proteins, which includes Gc-globulin, albumin, α-fetoprotein, and afamin *(1)*. One well-recognized function of Gc-globulin is to act as a carrier protein for vitamin D and its plasma metabolites, but Gc-globulin can also be converted into a macrophage-activating factor, known as Gc-globulin-MAF or Gc-MAF, through partial deglycosylation.

One of the intriguing properties of Gc-globulin is its ability to bind extracellular actin released by necrotic cell destruction into the circulation and, thus, to protect against disseminated intravascular coagulation induced by polymerized forms of actin *(2)*. In this review we summarize the synthesis, structure and variants, multiple functional roles, analytical aspects, clinical relevance, and applications of Gc-globulin and of the actin-scavenging system.

## Synthesis, Structure, and Variants

The Gc-globulin gene is expressed in a wide variety of tissues *(3)*, and the protein is present in various body fluids *(1)*. The vast majority of serum Gc-globulin is derived from expression and secretion by liver parenchymal cells, but minor contributions by nonhepatic cell types and nonparenchymal liver cells (our unpublished observation) exist. Gc-globulin has also been shown to be attached to the surface of a large number of cells *(4)*, although they do not produce it. Together with the genes that encode albumin, α-fetoprotein, and the tocopherol-binding protein afamin, the Gc-globulin gene is located on chromosome 4, sublocalized to bands 4q11-q13. Gc-globulin contains 458 amino acids, folded into a disulfide-bonded, triple-domain structure divided into 2 repeated, homologous domains of 186 amino acids (domains I and II) and a shorter domain of 86 residues at the COOH terminus (domain III). Two binding regions have been identified within the sequence: a vitamin D–binding domain localized between residues 35 and 49 (domain I) and an actin-binding domain between residues 373 and 403 (domains II and III). The actin-binding site does not appear to interact significantly with vitamin D binding *(5)*. Currently, it is not known in detail whether the

[1] Institute of Clinical Chemistry and Pathobiochemistry and Central Laboratory, Rheinisch-Westfälische Technische Hochschule (RWTH)–University Hospital Aachen, Aachen, Germany.

[2] Department of Internal Medicine I, University of Bonn–University Hospital Bonn, Bonn, Germany.

* Address correspondence to this author at: Institute of Clinical Chemistry and Pathobiochemistry and Central Laboratory, Rheinisch-Westfälische Technische Hochschule (RWTH)–University Hospital Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany. Fax 49-241-8082512; e-mail umeier@ukaachen.de.

Received December 21, 2005; accepted May 2, 2006.

Previously published online at DOI: 10.1373/clinchem.2005.065680

[3] Nonstandard abbreviations: Gc, group-specific component; MAF, macrophage (and osteoclast)-activating factor; MODS, multiple organ dysfunction syndrome; ARDS, acute respiratory distress syndrome; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin $D_3$ (calcitriol); $25(OH)D_3$, 25-hydroxyvitamin $D_3$ (calcidiol); AHF, acetaminophen-induced acute hepatic failure; and COPD, chronic obstructive pulmonary disease.

binding capacity and/or affinity of Gc-globulin for G-actin is influenced by the loading with vitamin D and its metabolites. The actin-scavenging characteristics of Gc-globulin therefore cannot be predicted from the transport function of this protein in vitamin D metabolism. Only a minority of 5% of total plasma Gc-globulin is occupied by vitamin D, leaving the majority free for scavenger functions *(1)*.

There are 3 codominant isoforms of full-length Gc-globulin, known as Gc1s, Gc1f, and Gc2, which differ by amino acid substitutions and glycosylation patterns *(6)*. In addition to these 3 common isoforms, more than 120 genetic variants have been described. Gc2 is most prevalent among Caucasians but is rare in Africans, whereas the opposite is true for Gc1f. Gc-globulin variants are associated with different plasma concentrations of Gc-globulin, being relatively highest in Gc1-1 (Gc1s-1s, Gc1s-1f, and Gc1f-1f), intermediate in Gc1-2 (Gc1s-2 and Gc1f-2), and lowest in Gc2-2 *(7)*.

## Actin-Scavenging Functions

The function of Gc-globulin in trauma is associated with its role as an actin-scavenging protein in the vascular and extracellular system (Fig. 1). To date, Gc-globulin has been recognized widely as a protein with markedly decreased concentrations in inflammatory and necrotic diseases.

Actins are highly conserved proteins that are part of the cytoskeleton. They are involved in cell motility and in maintenance of cell shape. The intracellular proteins are released into the systemic circulation after disruption of the cell membrane as a result of necrosis. In the plasma, actin can form long filaments (F-actin) together with coagulation factor Va, a condition that triggers disseminated intravascular coagulation if not rapidly resolved. Decompensation leads to a condition resembling multiple organ dysfunction syndrome (MODS) *(8)*. Thus, a complex actin-scavenging system has evolved in the vascular compartment, which involves 2 proteins: Gc-globulin and

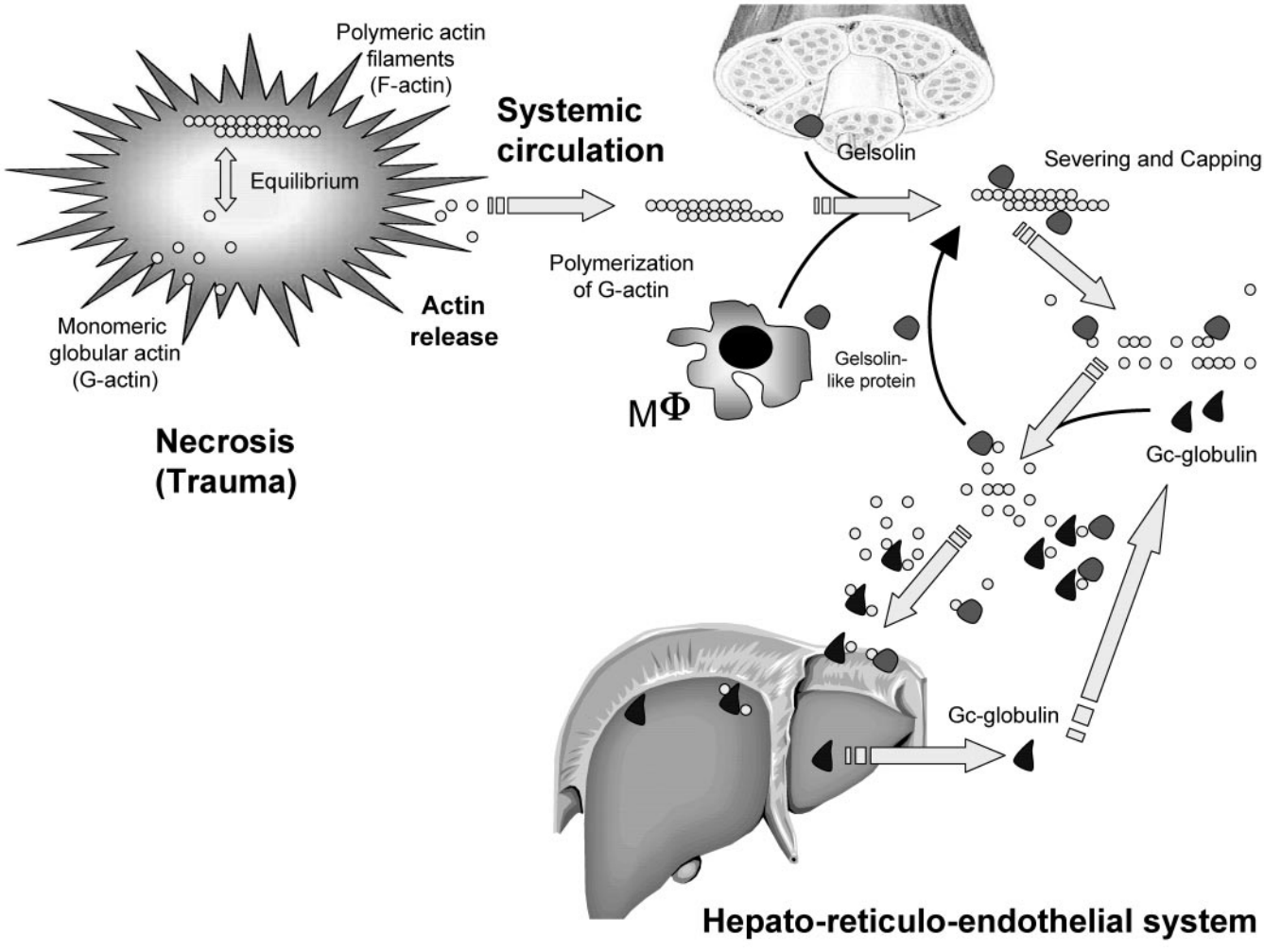


Fig. 1. The actin-scavenging system in the vascular and extracellular compartment involves 2 proteins: Gc-globulin and gelsolin.

gelsolin (Fig. 1). Gc-globulin binds to monomeric G-actin, which is released by the action of gelsolin, a protein composed of 6 homologous domains (G1–G6). During the scavenging process, both G-actin–gelsolin and G-actin–Gc-globulin complexes are formed and subsequently cleared by the reticuloendothelial system much more efficiently than the free proteins, which have half-lives of ~1 day (Gc-globulin) and 2 days (gelsolin), respectively. Studies in animals have revealed that the half-life of G-actin–Gc-globulin complex in vivo is ~30 min. The net result is that both gelsolin and Gc-globulin are consumed *(9)*. The plasma concentrations of Gc-globulin and gelsolin in a rat burn model *(10)* and in gelsolin-deficient mice *(11)* resemble those after severe injury. The finding that admission concentrations of gelsolin are also decreased in traumatized patients was supported in a study focusing on the role of gelsolin and the development of acute respiratory distress syndrome (ARDS) *(12)*.

Low total and actin-free Gc-globulin concentrations, the latter being an index of residual actin-scavenging capacity, have been demonstrated to be prognostic markers in situations of severe organ damage, such as fulminant hepatic failure *(13, 14)*, acetaminophen (paracetamol) overdose *(15)*, multiple trauma *(16)*, and multiple organ failure *(8, 17)*. Other clinical situations such as septic conditions have also been shown to be associated with low Gc-globulin concentrations and actin complexation *(16)*, but the specific role of Gc-globulin has yet to be defined.

Actin participates in many protein–protein interactions, including self-association of actin to form F-actin. A hydrophobic cleft between actin subdomains 1 and 3 is a "hot spot" for actin-binding proteins. Gc-globulin, F-actin, gelsolin, and ciboulot, a Wiskott–Aldrich syndrome protein family member, contain $\alpha$-helices that are characterized by the presence of exposed and conserved hydrophobic side chains that bind in the cleft of actin. This cleft has global hydrophobicity. A hydrophobic pocket in subdomain 1, located at the entrance of the hydrophobic cleft, appears to be a primary target for both G-actin– and F-actin–binding proteins, including Gc-globulin *(18)*.

### GELSOLIN

Gelsolin ($M_r$ 85 000) is an actin-binding protein that is primarily involved in gel-to-sol transformations. Gelsolin exists in cytoplasmic and secreted forms *(19)*. The skeletal muscle is the primary source of plasma gelsolin, although a gelsolin-like protein is also secreted by macrophages *(20)* (Fig. 1).

In the presence of $Ca^{2+}$, gelsolin severs and caps polymeric actin filaments (F-actin) *(21)*: after binding of domain G1 and partly domain G2 to the actin filament (F-actin), gelsolin rapidly severs F-actin and then remains bound to the barbed end of one of the newly formed filaments, forming a stable cap, thus inhibiting addition of further monomers. The resulting G-actin monomers are bound by Gc-globulin, which releases gelsolin from actin. Severing and capping are additionally regulated by specific interactions between gelsolin and polyphosphoinositides, which inhibit binding to actin *(22)*.

The concentration of gelsolin seems to be rate limiting in the actin removal system and depends on the rates of formation, dissociation, and clearance of the actin–gelsolin complex.

## Functions Other than Actin Scavenging

In addition to scavenging of actin, Gc-globulin also plays additional roles in some non–actin-scavenging functions (Fig. 2.).

### Gc-globulin as a transporter for vitamin d and endotoxin

Gc-globulin is a key component in the operation of vitamin D metabolism. It binds vitamin D and vitamin D analogs with different affinities at a binding site located in the N-terminal region of domain I *(23)*. Systemic transport of 1,25-dihydroxyvitamin $D_3$ [$1,25(OH)_2D_3$] and other dihydroxylated metabolites to target organs is managed by Gc-globulin. The dihydroxylated metabolites, particularly $1,25(OH)_2D_3$, bind to a nuclear receptor at the target cells, followed by subsequent generation of appropriate biological responses. Plasma clearance of Gc-globulin does not appear to be altered by 25-hydroxyvitamin $D_3$ [$25(OH)D_3$] occupancy. The binding affinities of Gc-globulin for vitamin D metabolites differ, providing differential availability of "free" (protein-unbound) steroids. For $1,25(OH)_2D_3$, which binds with lower affinity than $25(OH)D_3$, the fraction that exists as the free steroid is 0.4% of the total concentration, compared with 0.04% of total for $25(OH)D_3$. Other tasks of Gc-globulin, such as actin binding, alter neither its binding capacity nor its affinity for vitamin D steroids.

Gc-globulin also transports endotoxin. A clinical role of this binding activity has been suggested in patients with peritonitis because Gc-globulin concentrations could predict organ failure in this condition *(24)*. Thus, Gc-globulin could be an important scavenger of endotoxin in situations of endotoxinemia, such as septic conditions.

### Response in tissue injury or infection

There are 3 major Gc-globulin responses in tissue injury *(25)*: One response is in the actin-scavenging system, working in concert with gelsolin, as described above. Another response involves the release by activated T and B cells of enzymes that process O-linked carbohydrate side chains of Gc-globulin, transforming the molecule into Gc-MAF. In turn, Gc-MAF stimulates macrophage activity at the tissue site. The immune response is controlled by Gc-MAF, inducing apoptosis in activated macrophages via caspase induction. The third response of Gc-globulin to tissue injury is to bind to circulating neutrophils, which stimulates chemotactic localization to specific tissue sites. Additional functions include the inhibition of actin-induced platelet aggregation *(26)* and the stimulation of

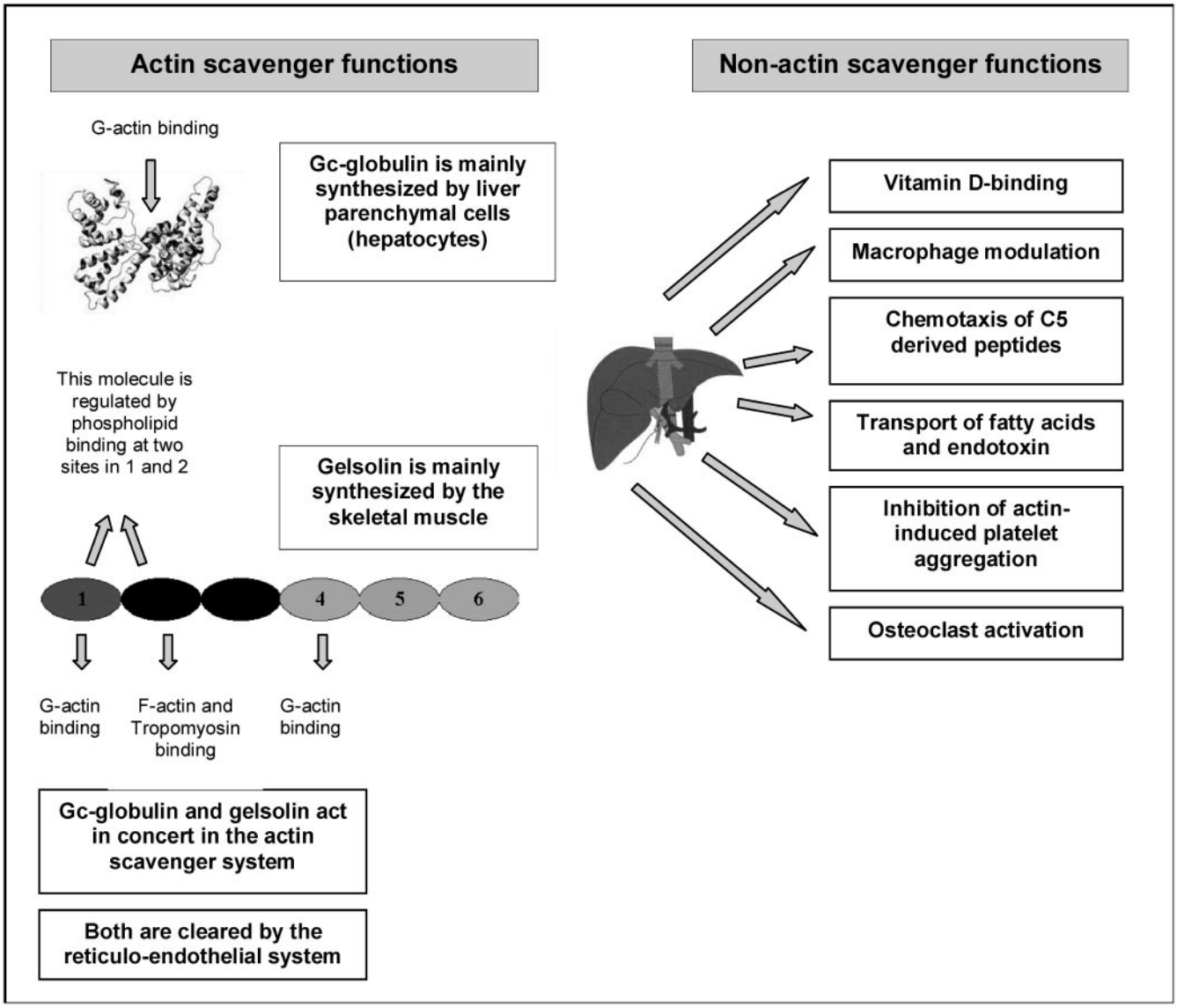


Fig. 2. Actin-scavenging and non–actin-scavenging functions of Gc-globulin.
Gelsolin also acts in the actin-scavenging system.

osteoclast activity and bone resorption by Gc-MAF *(27)*. All of these findings strongly suggest that Gc-globulin exerts more complex physiologic functions than clearance of actin released by necrotic cells and tissues from the circulation.

### Gc-globulin and chemotactic activity of complement factor 5–derived peptides

Gc-globulin exerts immunomodulatory functions through enhancing complement factor 5 (C5)-mediated signaling. Enhancement of C5a-dependent chemotactic activity by Gc-globulin has been observed in several cell types, primarily monocytes and neutrophils *(28)*. C5a elicits multiple effects on target cells, i.e., chemotaxis, primarily interacting with the cell-associated form of Gc-globulin present on the plasma membrane of diverse cell types *(1)*.

Because Gc-globulin is found in high concentrations in plasma as well as several other body fluids, including breast milk, seminal fluid, cerebrospinal fluid, saliva, and bronchoalveolar lavage fluid of patients with chronic obstructive pulmonary disease, this interaction is pathophysiologically relevant, indicating that Gc-globulin enhances the recruitment of immunocompetent cells to inflammatory foci via activation of C5a-mediated signaling *(29)*. Additional evidence for Gc-globulin as a major serum factor for chemotactic enhancement is provided by experiments demonstrating that Gc-globulin–depleted serum lacks chemotactic enhancing activity for C5a.

Both the binding of Gc-globulin to C5a and the generation of C5a cochemotactic activity show a slightly delayed response to inflammation (maximum activity is observed at <45 min) *(30)*, which is most likely attribut-

able to the expression of proteases that modify the Gc-binding site or Gc-globulin itself. A direct interaction of Gc-globulin with the C5a receptor as explanation for its cochemotactic effect was excluded by experiments showing that Gc-globulin does not alter the affinity of C5a for the C5a receptor *(29)*. After maximum activity is reached, degradation of Gc-globulin by activated neutrophils is observed only after 2 to 3 h, indicating that prolonged binding of Gc-globulin is a prerequisite for the generation of cochemotactic activity. Other studies showed that neutrophil elastase and thrombospondin-1 might effectively control the binding of Gc-globulin to neutrophils *(31)*.

Zhang and Kew *(32)* localized a C5a/C5adesArg-binding region in the Gc-globulin protein between amino acid residues 126 and 175. This common sequence and chemotactic activity are identical among the 3 major isoforms of Gc-globulin (Gc-1F, Gc-1S, and Gc-2). Circulating Gc-globulin colocalizes with both CD44 and annexin A2 receptors on the cell surface and may use both pathways to enhance C5a-mediated chemotaxis *(33)*. Annexin A2 and CD44 are receptors involved in cell movement *(34, 35)*. The annexin A2 receptor also associates with the cell-surface vitamin D [1,25$(OH)_2D_3$] receptor in lipid rafts *(36, 37)*, and it has been hypothesized that the interaction is essential for the endocytotic uptake of vitamin D. CD44 and annexin A2 receptors are widely expressed on the surface of many cell types, including most leukocytes, and thus are targets for Gc-globulin to mediate its systemic effects *(34, 35)*. This enables Gc-globulin to function any time C5a is generated.

## Immunoassay Methods for Gc-Globulin Measurement

The concentration of Gc-globulin can be determined as total Gc-globulin (actin-free and actin-complexed Gc-globulin) and as actin-free Gc-globulin.

Serum concentrations of total Gc-globulin range from ~200 to 600 mg/L in healthy donors. The concentration of total Gc-globulin is measured by simple immunoassay techniques with polyclonal or monoclonal antibodies (Table 1). However, the choice of method depends on the apparatus, reagents, and experience of the laboratory concerned.

Gelsolin is measured by nephelometry (range, 151–621 mg/L in healthy donors) *(9)*. The determination of actin-free Gc-globulin is more complex. Most published methods make use of rocket immunoelectrophoresis *(38)* and polyacrylamide gel electrophoresis (without sodium dodecyl sulfate) and semiquantification by immunoblotting *(39)* (Table 1). These are time-consuming, indirect, and relatively imprecise procedures, taking more than 1 working day to obtain results.

A direct way of measuring actin-free Gc-globulin is by a rapid sandwich ELISA (Dianova). Actin-free Gc-globulin bound to the coat of a monoclonal antibody that is capable of binding Gc-globulin, free or complexed with actin, is detected with a second labeled monoclonal antibody that specifically binds to epitopes of the actin-free Gc-globulin. Thus, only actin-free Gc-globulin is measured, with an assay time of <1 h. The selective detection of actin-free Gc-globulin in human serum makes it possible to trace the free Gc-globulin reserve in critically ill patients in a short turnaround time during their clinical course. The reference values for actin-free Gc-globulin depend on the methods used (Table 1). Serum concentrations of actin-free Gc-globulin determined by the sandwich ELISA range from ~92 to 332 mg/L in healthy donors. The 2.5th centile value for actin-free Gc-globulin in healthy persons, as determined by the rapid sandwich ELISA, is ~100 mg/L. Values <100 mg/L should be regarded as potentially indicative of a reduced reserve of actin-free Gc-globulin.

## Clinical Relevance of Gc-Globulin

### TRAUMA

Various studies have demonstrated a direct correlation between serum concentrations of Gc-globulin and the survival rate of polytraumatized patients, showing that the Gc-globulin concentration is decreased shortly after trauma, largely because of its increased consumption within the actin-scavenging system. Measurement of the

**Table 1. Analysis of circulating Gc-globulin in disease conditions.[a]**

| | Total Gc-globulin | | | Actin-free Gc-globulin | | |
|---|---|---|---|---|---|---|
| **Disease** | **Concentration** | **Method(s)** | **Reference** | **Concentration** | **Method** | **Reference** |
| Healthy persons | 200–600 mg/L | Immunodiffusion, immunoturbidimetry, immunonephelometry, inhibition-ELISA | *(40)* | 92–332 mg/L | Sandwich ELISA[b] | |
| Fulminant hepatic failure | ≤100 mg/L | Rocket immunoelectrophoresis | *(17)* | ≤34 mg/L | PAGE[c] without SDS, quantified by immunoblotting | *(39)* |
| AHF[d] | ≤120 mg/L | Rocket immunoelectrophoresis | *(15)* | | | |
| Multiple trauma | ≤200 mg/L | Rocket immunoelectrophoresis | *(14)* | | | |

[a] Compiled from Refs. *(14, 15, 17, 39, 40)*.
[b] Determined by manufacturer.
[c] PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.
[d] Acetaminophen-induced.

Gc-globulin concentration after trauma can be used for early identification of patients with increased risk of lethality. Additional studies revealed that, in polytraumatized patients, concentrations of total and actin-free Gc-globulin are significantly higher in survivors than in nonsurvivors *(15, 17, 40)*.

### acute liver failure

Deficiency of Gc-globulin plays an important role in the pathophysiology of liver injury in both non–acetaminophen-induced acute hepatic failure (non-AHF) and AHF. The admission concentrations of serum Gc-globulin provide prognostic information that is precise enough to assess the course of the disease, with a reliability that is comparable to the Kings College criteria *(14)*. Because Gc-globulin is synthesized in the liver, hepatic insufficiency decreases serum concentrations of Gc-globulin, further aggravating the situation through impairment of the actin-scavenging system. Admission serum concentrations of total Gc-globulin were decreased by 75% in patients with AHF, and patients who had the most fulminant course had the lowest concentrations *(41, 42)*. The number of organ failures inversely correlated with serum concentrations of Gc-globulin *(13)*.

### sepsis

Several independent clinical studies *(16, 43)* have shown that low Gc-globulin concentrations are associated with a poor prognosis for survival and an increased risk of developing MODS in sepsis. This association is similar to that of traditional clinical risk factors, such as the number and kind of traumatized organs. Early decreases in circulating actin-free Gc-globulin were associated with the development of disseminated intravascular coagulation, whereas persistently low concentrations were correlated with the occurrence of ARDS *(16)*. The identification of Gc-globulin as an endotoxin-binding protein suggests its involvement in the clearance of this important septic mediator. Impairment of this function by decreases in the systemic concentration of Gc-globulin might promote the development of septicemia and, hence, could be deleterious for the patient.

## Clinical Applications

Gc-globulin has an array of biological activities and therefore a range of potential therapeutic applications *(25)*. Measurement of total Gc-globulin, and particularly of actin-free Gc-globulin, could aid in the management of patients with severe injury, so that decisions can be made whether to treat with plasma (a source of Gc-globulin) or Gc-globulin concentrate, if available. The knowledge of Gc-globulin concentrations can also contribute to decision-making for acute liver transplantation.

The macrophage-stimulating activities of Gc-globulin have led to applications, including inhibition of the growth of various types of cancer. Chronic obstructive pulmonary disease (COPD) is thought to have a genetic link, possibly involving the gene encoding Gc-globulin *(44)*. The authors concluded that the damage to the lung parenchyma might be related to the ability of Gc-globulin to be converted to Gc-MAF, which in turn increases macrophage stimulation. The presence of a homozygous Gc2 allele provided protection against the deleterious effects of chronic cigarette smoking *(44)*. This observation raises the possibility that a recombinant product based on Gc2 could serve as a means of ameliorating COPD.

It has been suggested that, in patients with advanced cancer, macrophages cannot be activated because cancer cells efficiently inactivate Gc-globulin. The cancer cells release an endoglycosidase (*N*-acetylgalactosaminidase) that inactivates Gc-MAF. Replacing Gc-MAF could restore the capability of macrophages to remove cancer cells *(45)* and could reverse the immunosuppression. The inactivation of Gc-MAF by a-*N*-acetylgalactosaminidase is also thought to be involved in the etiology of immune-suppressed conditions, such as HIV infection and systemic lupus erythematosus *(46)*. Treatment of these patients with Gc-MAF to stimulate macrophages could offer a means of clearing immune complexes. These data provide evidence that Gc-MAF might have a spectrum of activities relevant for the treatment of cancer by improving immune surveillance.

Evaluations of the modulation of Gc-globulin concentrations under specific disease conditions could be included in future therapeutic trials and may further support the relevance of this intriguing protein in clinical medicine from both the diagnostic and therapeutic points of view.

## References


1. White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab 2000; 11:320–7.
2. Lee WM, Galbraith RM. The extracellular actin-scavenger system and actin toxicity. N Engl J Med 1992;326:1335–41.
3. McLeod JF, Cooke NE. The vitamin D-binding protein, $\alpha$-fetoprotein, albumin multigene family: detection of transcripts in multiple tissues. J Biol Chem 1989;264:21760–9.
4. DiMartino SJ, Kew RR. Initial characterization of the vitamin D binding protein (Gc-globulin) binding site on the neutrophil plasma membrane: evidence for a chondroitin sulfate proteoglycan. J Immunol 1999;163:2135–42.
5. Haddad JG, Hu YZ, Kowalski MA, Laramore C, Ray K, Robzyk P, et al. Identification of the sterol- and actin-binding domains of plasma vitamin D binding protein. Biochemistry 1992;31:7174–81.
6. Braun A, Bichlmaier R, Cleve H. Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. Hum Genet 1992;89:401–6.
7. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. Clin Chem 2001;47:753–6.
8. Dahl B. The extracellular actin scavenger system in trauma and major surgery. Clinical and experimental studies. Acta Orthop Suppl 2005;76:1–24.

**9.** Dahl B, Schiødt FV, Ott P, Gvozdenovic R, Yin HL, Lee WM. Plasma gelsolin is reduced in trauma patients. Shock 1999;12:102–4.

**10.** Rothenbach PA, Dahl B, Schwartz JJ, O'Keefe GE, Yamamoto M, Lee WM, et al. Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. J Appl Physiol 2004;96:25–31.

**11.** Becker PM, Kazi AA, Wadgaonkar R, Pearse DB, Kwiatkowski D, Garcia JG. Pulmonary vascular permeability and ischemic injury in gelsolin-deficient mice. Am J Respir Cell Mol Biol 2003;28:478–84.

**12.** Mounzer KC, Moncure M, Smith YR, DiNubile MJ. Relationship of admission gelsolin levels to clinical outcomes in patients after major trauma. Am J Respir Crit Care Med 1999;160:1673–81.

**13.** Schiødt FV, Ott P, Bondesen S, Tygstrup N. Reduced serum Gc-globulin concentrations in patients with fulminant hepatic failure: association with multiple organ failure. Crit Care Med 1997;25:1366–70.

**14.** Schiødt FV, Bondesen S, Petersen I, Dalhoff K, Ott P, Tygstrup N. Admission levels of serum Gc-globulin: predictive value in fulminant hepatic failure. Hepatology 1996;23:713–8.

**15.** Schiødt FV, Ott P, Tygstrup N, Dahl B, Bondesen S. Temporal profile of total, bound, and free Gc-globulin after acetaminophen overdose. Liver Transpl 2001;7:732–8.

**16.** Dahl B, Schiødt FV, Ott P, Wians F, Lee WM, Balko J, et al. Plasma concentration of Gc-globulin is associated with organ dysfunction and sepsis after injury. Crit Care Med 2003;31:152–6.

**17.** Dahl B, Schiødt FV, Rudolph S, Ott P, Kiaer T, Heslet L. Trauma stimulates the synthesis of Gc-globulin. Intensive Care Med 2001;27:394–9.

**18.** Dominguez R. Actin-binding proteins—a unifying hypothesis. Trends Biochem Sci 2004;29:572–8.

**19.** Kwiatkowski DJ, Mehl R, Yin HL. Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. J Cell Biol 1988;106:375–84.

**20.** Johnston PA, Yu FX, Reynolds GA, Yin HL, Moomaw CR, Slaughter CA, et al. Purification and expression of gCAP39. An intracellular and secreted $Ca^{2+}$-dependent actin-binding protein enriched in mononuclear phagocytes. J Biol Chem 1990;265:17946–52.

**21.** McGough A, Chiu W, Way M. Determination of the gelsolin binding site on f-actin: implications for severing and capping. Biophys J 1998;74:764–72.

**22.** Silacci P, Mazzolai L, Gauci C, Stergiopulos N, Yin HL, Hayoz D. Gelsolin superfamily proteins: key regulators of cellular functions. Cell Mol Life Sci 2004;61:2614–23.

**23.** Swamy N, Head JF, Weitz D, Ray R. Biochemical and preliminary crystallographic characterization of the vitamin D sterol- and actin-binding by human vitamin D-binding protein. Arch Biochem Biophys 2002;402:14–23.

**24.** Berger D, Kitterer WR, Berger HG. Are the serum levels of endotoxin-binding proteins reliable predictors of complications in the course of peritonitis? Eur J Clin Invest 1990;20:66–71.

**25.** Gomme PT, Bertolini J. Therapeutic potential of vitamin D-binding protein. Trends Biotechnol 2004;22:340–5.

**26.** Vasconcellos CA, Lind SE. Coordinated inhibition of actin-induced platelet aggregation by plasma gelsolin and vitamin D-binding protein. Blood 1993;82:3648–57.

**27.** Schneider GB, Grecco KJ, Safadi FF, Popoff SN. The anabolic effects of vitamin D-binding protein-macrophage activating factor (DBP-MAF) and a novel small peptide on bone. Crit Rev Eukaryot Gene Expr 2003;13:277–84.

**28.** Piquette CA, Robinson-Hill R, Webster RO. Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin. J Leukoc Biol 1994;55:349–54.

**29.** Perez HD. Gc globulin (vitamin D-binding protein) increases binding of low concentrations of C5a des Arg to human polymorphonuclear leukocytes: an explanation for its co-chemotaxin activity. Inflammation 1994;18:215–20.

**30.** Kew RR, Fisher JA, Webster RO. Co-chemotactic effect of Gc-globulin (vitamin D binding protein) for C5a. Transient conversion into an active co-chemotaxin by neutrophils. J Immunol 1995;155:5369–74.

**31.** Trujillo G, Kew RR. Platelet-derived thrombospondin-1 is necessary for the vitamin D-binding protein (Gc-globulin) to function as a chemotactic cofactor for C5a. J Immunol 2004;173:4130–6.

**32.** Zhang J, Kew RR. Identification of a region in the vitamin D-binding protein that mediates its C5a chemotactic cofactor function. J Biol Chem 2004;279:53282–7.

**33.** McVoy LA, Kew RR. CD44 and annexin A2 mediate the C5a chemotactic cofactor function of the vitamin D binding protein. J Immunol 2005;175:4754–60.

**34.** Rescher U, Gerke V. Annexins—unique membrane binding proteins with diverse functions. J Cell Sci 2004;117:2631–9.

**35.** Pure E, Cuff CA. A crucial role for CD44 in inflammation. Trends Mol Med 2001;7:213–21.

**36.** Mizwicki MT, Bishop JE, Olivera CJ, Huhtakangas J, Norman AW. Evidence that annexin II is not a putative membrane receptor for $1\alpha$,25(OH)2-vitamin D3. J Cell Biochem 2004;91:852–63.

**37.** Huhtakangas JA, Olivera CJ, Bishop JE, Zanello LP, Norman AW. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds $1\alpha$,25(OH)2-vitamin D3 in vivo and in vitro. Mol Endocrinol 2004;18:2660–71.

**38.** Goldschmidt-Clermont PJ, Galbraith RM, Emerson DL, Werner PA, Nel AE, Lee WM. Accurate quantitation of native Gc in serum and estimation of endogenous Gc: G-actin complexes by rocket immunoelectrophoresis. Clin Chim Acta 1985;148:173–83.

**39.** Lee WM, Galbraith RM, Watt GH, Hughes RD, McIntire DD, Hoffman BJ, et al. Predicting survival in fulminant hepatic failure using serum Gc protein concentrations. Hepatology 1995;21:101–5.

**40.** Jorgensen CS, Christiansen M, Norgaard-Pedersen B, Ostergaard E, Schiodt FV, Laursen I, et al. Gc globulin (vitamin D-binding protein) levels: an inhibition ELISA assay for determination of the total concentration of Gc globulin in plasma and serum. Scand J Clin Lab Invest 2004;64:157–66.

**41.** Schiødt FV, Rossaro L, Stravitz RT, Shakil AO, Chung RT, Lee WM. Gc-globulin and prognosis in acute liver failure. Liver Transpl 2005;11:1223–7.

**42.** Jalan R. Gc-globulin to predict outcome in acute liver failure: a panacea? Liver Transpl 2005;11:1169–71.

**43.** Lee CC, Marill KA, Carter WA, Crupi RS. A current concept of trauma-induced multiorgan failure. Ann Emerg Med 2001;38:170–6.

**44.** Ito I, Nagai S, Hoshima Y, Muro S, Hirai T, Tsukino M, et al. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. Chest 2004;125:63–70.

**45.** Kisker O, Onizuka S, Becker CM, Fannon M, Flynn E, D'Amato R, et al. Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. Neoplasia 2003;5:32–40.

**46.** Yamamoto N, Naraparaju VR, Moore M, Brent LH. Deglycosylation of serum vitamin $D_3$-binding protein by $\alpha$-*N*-acetylgalactosaminidase detected in the plasma of patients with systemic lupus erythematosus. Clin Immunol Immunopathol 1997;82:290–8.