Conglutinin – an Important Element of Natural Immunity in Ruminants (a Review)

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Abstract

Conglutinin represents a collectin appearing only in some mammals, including cattle. It plays an important role in resistance of hosts against microbes and parasites. It exhibits affinity toward saccharides present on the surface of bacteria, viruses, fungi and parasites and it mobilizes the mechanisms leading to their inactivation. It manifests also the unique ability of agglutinating complement- and antibody-coated erythrocytes. Moreover, it shows the ability to activate immune system cells, including the process of phagocytosis. In this review also the factors that determine the amount and activity of conglutinin in mammalian serum were presented, including environmental conditions and infectious agents, such as various types of bacteria and viruses. The broad scope of conglutinin is genetically controlled and, therefore, this collectin may constitute a useful and crucial factor in cattle husbandry

Conglutinin represents the first protein in the group of present collectins, detected in cattle serum by Ehrlich and Sachs in 1902 and by Bordet in 1909, participating in the reaction termed conglutination (agglutination of complement-coated erythrocytes) (Ingram and Barnum 1965). The phenomenon was registered and reported in cases of bacteria-infected cattle (Ingram and Mitchell 1971), including cattle infected with *Mycobacterium bovis* (Holmskov et al. 1998), in which decreased agglutinating activity was described. Conglutinin is accepted to represent an important element of non-specific humoral immunity, at present termed the inborn immunity, found only in some ruminants of the *Bovidae* family (Koch et al. 2001; Thiel et al. 2006). The substance was demonstrated to exhibit anti-microbial properties: a mutation present in genes coding protein was found to increase propensity of the animals to bacterial, viral, fungal and parasitic infections (Koch et al. 2001; Thiel et al. 2006). Its anti-microbial and anti-parasitic role has been linked to its affinity to saccharides present on the surface of microbes and parasites (Lachmann 1962; Lu 1997). The reaction leads to activation of complement which, together with binding of the immobilised complement by specific receptors on phagocytic cells, stimulates the process of phagocytosis (Lu 1997).

Structure of conglutinin

Conglutinin is a protein of around 100 to over 1000 kDa molecular weight (Hansen and Holmskov 1998). Its principal functional form ensuring its appropriate activity involves a trimer (Dec and Wernicki 2006). In various collectins the number of trimeric subunits may vary but conglutinin contains four trimeric subunits (Hansen et al. 2002). In each monomeric subunit of collectins the N-terminal cysteine-rich domain, the collagen-like domain and the α-helical neck domain plus C-type lectin domain can be distinguished, the latter termed also CRD (carbohydrate-recognition domain) (Eda et al. 1996; van der Wetering et al. 2004). Every trimer consisting of monomers is stabilised by inter-monomer covalent disulphide bridges, binding cystein residues in N-terminals or in collagen

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domains as well as by non-covalent interactions (Hoppe and Reid 1994). Moreover, in microscopical images the tetrameric molecule of conglutinin with two globular heads was found to be linked to the core by four elongated collagen branches (Andersen et al. 1992). The entire molecule contains 351 amino acid residues, including 55 Gly-X-Y repetitions with two intervals and the diameter of its tetramers is 96 nm (Lachmann 1967; Lee et al. 1991; Andersen et al. 1992; Hansen et al. 2002). The long collagen-like domains of the protein are separated by short NH$_2$-terminal regions, consisting of 25 residues, originating from the long (155 residues) globular COOH terminus (Lachmann 1967; Lee et al. 1991; Hansen et al. 2002). Structural analysis of conglutinin demonstrated that its structure contains also 7 cystein residues, capable of forming inter- and/or intra-chain disulphide bridges (Lachmann 1967; Hansen et al. 2002). Two of the residues are present in the non-collagenous N-terminal segment (residues 15 and 20), four in the non-collagenous COOH-terminal segment (residues 255, 327, 341 and 349) and one of them is present in the collagenous domain (residue 38) (Lee et al. 1991). In electron microscope the conglutinin molecule resembles in shape the letter X (Andersen et al. 1992; Lu et al. 1993).

Localization of biosynthesis sites and occurrence of conglutinin

The conglutinin-coding genes were found to be located in the chromosome 28 of cattle, at the q1.8 position (Gallagher et al. 1993; Liou et al. 1994; Hansen et al. 2002, Hansen et al. 2003), although the protein could also derive from collectin representing the SP-D, the surfactant protein D, which represents a sequel of total or partial duplication of primordial *SP-D* gene that occurred following divergence of the *Bovidae* family from other mammals (Lim et al. 1993; Holmskov 2000; Hansen et al. 2003). The hypothesis was confirmed by 78% identity of amino acid sequence between cattle collectins in the form of SP-D on one hand and conglutinin on the other (Lim et al. 1993). Presence of conglutinin was detected only in ruminants, such as cattle, dromedary, *Kobus ellipsiprymnus*, kobus from Uganda, kobus from Kenia, dikdik antelope (*Madoqua saltiana*), *Damaliscus lunatus*, hartebeest (*Alcelaphus buselaphus*), in which titre of conglutinin is particularly high (Kakoma and Kinyanjuni 1974; Dec and Wernicki 2006). Conglutinin is present in their serum, neutrophils, follicular dentritic cells, macrophages originating from liver, lungs and spleen, in thymus, lymph nodes and tonsils (Holmskov et al. 1992; Lim et al. 1993; Lu et al. 1993; Hansen et al. 2002). The localization indicates that conglutinin plays an important role in the immune system, particularly because of its ability to direct the binding of saccharide residues on the surface of microbes which promotes phagocytosis and antigen presentation (Holmskov et al. 1992).

Biological properties of conglutinin

This biologically very specific protein represents a heat-stable beta-globulin, synthesized by cells of the immune system, i.e., by neutrophils (Deptuła et al. 2008), but also and probably in prevailing amounts by hepatocytes (Lu et al. 1993). Its unique property of agglutinating erythrocytes pre-incubated with antibodies and complement (Strang et al. 1986; Holmskov et al. 1992; Lu et al. 1993; Laursen et al. 1994, Dec and Wernicki 2006), depends on the presence of calcium and serum conglutinin activity factor (CAF), termed also the factor I (Eda et al. 1996). The factor splits C3b component of the complement, forming an inactive iC3b factor, which opsonizes erythrocytes (Strang et al. 1986; Hartley et al. 1992; Wakamiya et al. 1992; Hartshorn et al. 1993; Laursen et al 1994). Long-term incubation of antibody-coated erythrocytes with serum, preceded by incubation with conglutinin, abrogates the agglutinating properties (Laursen et al. 1994), due to the splitting of iC3b to C3d and C3c (Linscott et al. 1978; Ross et al. 1982; Laursen et al. 1994). Binding of conglutinin to iC3b was demonstrated to be calcium-dependent but

may also be inhibited by monosaccharides, of which N-acetyl-D-glucosamine (GlcNAc) exerts a particularly strong effect (Laursen et al. 1994). Conglutinin demonstrates also affinity to oligosaccharides in the α chain of iC3b (Lu et al. 1993; Laursen et al. 1994), and manifests capacity to bind N-acetylglucosamine, mannose and fructose residues (Lee et al. 1991). *In vitro,* the protein was found to opsonize *Salmonella typhimurium* and *Escherichia coli* (Holmskov et al. 1998), although the ability was dependent on the presence of an intact complement system and, hence, serum devoid of CAF did not promote the reaction (Holmskov et al. 1998). Studies of Friis-Christiansen et al. (1990) showed that conglutinin binding iC3b localized on bacterial surface caused that also bacteria coated in this way "cooperated" with macrophages (Holmskov et al. 1998).

Conditions which affect the amount and activity of conglutinin in mammals

The amount of conglutinin in serum of cows reflects several conditions, including environmental and infectious variables (Ingram and Barnum 1965; Ingram and Mitchell 1970; Ingram and Mitchell 1971; Du Plessis 1985; Krogh-Meibom et al. 2004; van der Wetering et al. 2004; Thiel et al. 2006). In the serum of adult individuals (studies conducted mainly on Holstein cows), its titre reached the value of 0 to 5 120 (Ingram and Barnum 1965, Thiel et al. 2006). Such broad range of conglutinin concentrations in serum is genetically conditioned (Holmskov et al. 1998). Therefore, it is assumed that high inheritance of conglutinin concentrations provides a valuable and significant index, which is also taken into account in cattle husbandry since estimation of the content allows selecting individuals with higher resistance to diseases (Holmskov et al. 1998). Serum of newly born calves contains no conglutinin until the moment of colostrum administration (Ingram and Barnum 1965; Ingram and Mitchell 1970). Following the first administration of colostrum, the amount of conglutinin increases until the 20th month of life, i.e., until the concentration is attained that is present in adult cattle (Ingram and Barnum 1965; Ingram and Mitchell 1970). The concentration was also found to depend on the sex and breed of cattle (Holmskov et al. 1998). Also the season of the year and environmental/feeding conditions were found to affect the concentration of conglutinin (Ingram and Barnum 1965; van der Wetering et al. 2004): low amount of conglutinin was observed in late winter and in spring, its high level could be detected in fall and early winter. A very marked increase in its concentration in serum could be noted within few weeks after the first pasture in the field. The high serum concentration of conglutinin in the animals was also found to reflect environmental temperatures and intensity of day light (Ingram and Barnum 1965), but the concentration was not affected by physiological rhythms (Krogh-Meibom et al. 2004). Conglutinin concentration was also strongly affected by pregnancy and abortion due to infections (Ingram and Barnum 1965; Ingram and Mitchell 1970; Ingram and Mitchell 1971). A clear-cut decrease in serum conglutinins in cows developed shortly before calving, starting at 4 weeks before term and persisting up to 2 weeks after delivery. Later, the level rapidly increased reaching high concentrations in approximately the 21st week after delivery (Ingram and Mitchell 1970). It was concluded that the situation is linked to sex hormones and/or corticosteroids (Ingram and Mitchell 1971). A decrease in conglutinin titre was recorded in the week of abortion or between the 2nd and 4th week after abortion, when no conglutinins were observed in serum (Ingram and Mitchell 1971).

Activity of conglutinin

The abortions due to infections and such inflammatory conditions as pneumonia, metritis or acute infections reduce titre and activity of conglutinin in calf serum (Ingram and Mitchell 1971). An important decrease in conglutinin titre developed already one week

before other morbid signs and persisted for 1-2 weeks after the infection took place (Ingram and Mitchell 1971a). The lowered conglutinin titres were recorded also in cows experimentally infected with *Cowdria ruminantium* or infected with *Anaplasma marginalne* (Rose et al. 1978; Du Plessis 1985). It was suggested (Dec and Wernicki 2006) that the reason for decreased conglutinin titres may involve marked hyperthermia, recorded in the infected cattle even if the probable cause of the decrease, mainly in the course of acute infections, may involve absorption of conglutinin from the serum by the activated complement component iC3b or a direct effect of microbial activity. Such conditions may arise also due to decreased activation thresholds of expression mechanisms of appropriate genes (Ingram and Mitchell 1971). Local infections, e.g., benign inflammation of mammary glands or contagious infection of hoofs (whitlow) in cattle do not considerably affect serum conglutinin amount (Ingram and Mitchell 1970). Also, no changes in their amount were noted in cattle in the course of lymphosarcoma (Ingram and Mitchell 1971). Nevertheless, experimental bacterial infections demonstrated that conglutinin remains an important variable in the infections, as noted already in 1959 by Ingram (1959). The author recorded reciprocal relationships between conglutinating activity of animal serum and experimentally induced bacterial infections. He found that pretreatment of mice with conglutinin secured higher proportions of surviving mice following their infection with virulent strains of *Salmonella typhimurium*, *Pasteurella septica*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Streptococcus pyogenes* (Ingram 1959). Moreover, it was noted that infection of the mice with *Salmonella typhimurium* resulted in an almost immediate reduction in amounts of conglutinins in serum, which could not be documented in experimental animals infected with pathogenic and non-pathogenic strains of *E. coli* (Ingram 1959a). Results of the studies related to *Salmonella* are consistent with those originating from studies in the 1990s (Friis-Christiansen 1990), in which mice subcutaneously injected with conglutinin manifested extended survival following intraperitoneal injection of a highly virulent strain of *S. typhimurium*. Moreover, the animals manifested considerably reduced numbers of bacteria in blood in comparison to control animals administered saline (Friis-Christiansen 1990). The authors demonstrated that the anti-bacterial activity of conglutinin directed to *Salmonella typhimurium* was pre-conditioned not only by the presence of the complement system but also macrophages. They hypothesized that, binding to bacterial surface anchored iC3b, conglutinin acts as a ligand mediating contact between the bacteria and the cells. They also suggested that activation of macrophages is probably linked to a mechanism of conglutinin stimulating special receptors of the cell surface which result in formation of oxygen metabolites in the cells and in this way it induces their anti-microbial activity (Friis-Christiansen 1990).

Conglutinin was also found to exhibit anti-viral activity: its activity which inhibits virally-induced haemagglutination due to aggregation of viruses abolishes infectivity of the viruses, which has been registered in the case of group A influenza viruses, subtypes H1, H2 and H3 (Hartley et al 1992; Wakamiya et al. 1992; Hartshorn et al. 1993). Due to its lectin properties, the collectin neutralizes also other viruses (Eda et al. 1996). The latter property was documented toward rotaviruses, the VP7 protein which was bound by lectin domains of conglutinin (Ramig 2004). Such a binding results in inhibited haemagglutination by rotaviruses and in abolished infectivity of the viruses, which, as claimed by Reading et al. (1998), also depends on glycosylation of VP7 protein. This was shown studying *in vitro* the anti-viral activity of conglutinins directed toward bovine rotavirus NCDV (Nebraska calf diarrhoea virus), demonstrating that the protein neutralised the rotavirus by binding its VP7 glycoprotein through lectin domains of conglutinin. The potential of conglutinin was also demonstrated to bind *Herpes simplex type 2* (HSV2) viruses in presence of calcium ions (Fisher et al. 1994). In those studies the protein was administered to mice before vaccine in order to protect against infection with *Herpes* virus, accentuated viral replication indicating

that the vaccine provided another place for the virus to penetrate host cells (Fisher et al. 1994). In other studies (Rose et al. 1978) binding of conglutinin with HSV2 virus did not neutralize infectivity of the virus. Binding of conglutinin was demonstrated also in the case of HIV: in presence of calcium ions conglutinin bound glycoprotein 160 of the viral envelope although the reaction was noted to be inhibited by N-acetyl-D-glucosamine and by deglycosylation of the viral gp160 protein (Andersen et al. 1991). Results of the studies demonstrated also that conglutinin may inhibit infections with HIV by blockade of interactions between CD4+ cells and the virus (Andersen et al. 1991).

It seems also interesting that no reasons are known for the absence of conglutinin in the sera of other herbivorous animals than ruminants, e.g. in the sera of horses that also live in symbiosis with enormous numbers of bacteria present in their caecum. No conglutinin could be detected in the sera of mice, rabbits, guinea pigs, cats, dogs, pigs (Rose et al. 1978), although its small amounts were noted in the sera of humans and rats, even if it was not purified and characterised (Lachmann 1967; Holmskov 1992).

Conclusion

Independently of the latter facts, conglutinin can be concluded to represent an important factor of non-specific humoral or innate immunity in animals of *Bovidae* family. It participates in immune processes allowing protecting hosts from various pathogens, including bacteria and viruses and, manifesting capacity to aggregate and neutralize viruses, promoting activation of the immune system including phagocytosis.

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