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MINIREVIEW

Calprotectin – a Pleiotropic Molecule in Acute and Chronic Inflammation

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Summary

Calprotectin (MRP8/14, S100A8/S100A9, 27E10 antigen) is a heterodimer of two calcium-binding proteins present in the cytoplasm of neutrophils and expressed on the membrane of monocytes. Upon neutrophil activation or endothelial adhesion of monocytes, calprotectin is released and may be detected in serum or body fluids as potentially useful clinical inflammatory marker. The soluble form of calprotectin provides both bacteriostatic and cytokine-like effects in the local environment. When calprotectin metabolism is affected on a systemic level, the zinc-binding properties of protein may induce severe dysregulation of zinc homeostasis with severe clinical symptoms. The distribution of membrane form of calprotectin is restricted to monocytes and immature macrophages and the presence of calprotectin-positive infiltrating cells reflects the influx of mononuclear phagocytes to the site of inflammation. Calprotectin expression and release seems to be of particular importance in immune and immunopathological reactions.

Key words
Calprotectin • MRP8/14 • S100A8 • S100A9 • Inflammation • Neutrophils

"Vestigia terrent" Horatius

Calprotectin was originally discovered as an antimicrobial protein that was present in the cytoplasm of neutrophil granulocytes (Dale *et al.* 1983). Subsequently, it has been recognized as a promising marker of inflammation, or rather a trace of the antagonism going on inside the organism (Sander *et al.* 1984, Roth *et al.* 2001). Furthermore, the molecule is involved in the recruitment of inflammatory cells by interactions with endothelial cells (Srikrishna *et al.* 2001) and its zinc-capturing function may affect physiological homeostasis (Sampson *et al.* 2002). The pleiotropic functions of calprotectin are associated mostly with active inflammatory processes including antibacterial defense mechanisms or with Th1-mediated responses such as allograft rejection or in autoimmune reactions.

Calprotectin structure

Calprotectin can be found in the literature under several synonyms (complex of S100A8 and S100A9 proteins, 27E10 antigen, macrophage inhibitory factor-related protein MRP8/14, L1L and L1H proteins,

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calgranulin A/B). It is a 24 kD heterodimer composed of light (MRP8) and heavy (MRP14) chains (8 and 14 kDa) (Bhardwaj *et al.* 1992, Hunter and Chazin 1998), members of the S-100 family (Kligman and Hilt 1988) of calcium-binding proteins (Steinbakk *et al.* 1990). The binding of calcium induces conformational changes, the calcium-saturated state, which allows binding of other proteins (Lewit-Bentley and Rety 2000). In the presence of calcium, MRP8/14 heterodimeric complexes may tetramerize into heterotetramers (Strupat *et al.* 2000). Calprotectin also contains zinc-binding domains, which have a zinc-binding capacity higher than other S100 proteins, and are not affected by the binding of calcium. Both MRP8 and MRP14 contain histidine-based zinc-binding sequences (His-X-X-X-His motif), which are involved in the antibacterial activity of calprotectin (Loomans *et al.* 1998).

Table 1. Essential characteristics of calprotectin

Nomenclature	calprotectin, MRP8/14 protein, calgranulin, L1 protein, 27E10 antigen	(Bhardwaj *et al.* 1992; Brandtzaeg *et al.* 1987; Gebhardt *et al.* 2002; Roth *et al.* 2001)
Molecular weight	36 kD	(Zwadlo *et al.* 1986)
Structure	24 kD heterodimer (or 48 kD tetramer) of 2 calcium-binding proteins MRP8 and MRP14 (S100A8 and S100A9)	(Itou *et al.* 2002; Roth *et al.* 1994; Strupat *et al.* 2000)
Structural relationship	S100 proteins family	(Kerkhoff *et al.* 1998; Kligman and Hilt 1988; Lewit-Bentley and Rety 2000)
Distribution	neutrophils, monocytes, acute phase macrophages, invariably in endothelial and epithelial cells	(Bhardwaj *et al.* 1992; Brandtzaeg *et al.* 1987; Doussiere *et al.* 2002; Helbert *et al.* 2001; Pillay *et al.* 1998; Roth *et al.* 1993)
Functions	an important role in inflammatory processes by regulating the adhesion of myeloid cells to endothelium and extracellular matrix and, activation of effector cells (e.g. induction of CD11b), direct antibacterial effects by zinc- capturing, induction of CD11b	(Clohessy and Golden 1995; Eue *et al.* 2002; Mahnke *et al.* 1995; Newton and Hogg 1998; Sampson *et al.* 2002)

Distribution of calprotectin

Calprotectin was originally found in neutrophils and a subpopulation of mononuclear phagocytes (Table 1). The reactivity of monoclonal antibody 27E10 showed restricted distribution within the myeloid cell lineage with a weak variable expression also in endothelial and epidermal cells (Zwadlo *et al.* 1986). The concentration of calprotectin in neutrophils is abundant and constitutes about half (30-60 % according to various authors) of total cytosolic protein (Hessian *et al.* 1993). Calprotectin is secreted extracellularly from stimulated neutrophils (Boussac and Garin 2000) and monocytes (Rammes *et al.* 1997), or is released as a result of cell disruption or death (Voganatsi *et al.* 2001). After cell death, calprotectin is released into pus or abscess fluid together with microbicidal nucleohistones. Immunohistochemical studies confirmed the presence of calprotectin not only in neutrophils and reactive tissue macrophages, but also on the membrane of non-

keratinizing squamous epithelia, and, occasionally, in kidney tubules. Some mucosal epithelial cells express calprotectin in the cytoplasm constitutively (Brandtzaeg *et al.* 1987). The soluble form of calprotectin is found in plasma (reference range < 2 mg/l in healthy subjects), urine (its production by kidney cells could prevent formation of calcium oxalate stones) (Pillay *et al.* 1998), body secretions (higher calprotectin levels were found in saliva of subjects with candidiasis, and the calprotectin concentration correlated positively with the severity of candidal infection), intestinal fluid and feces.

Physiological role of membrane calprotectin

The role of calprotectin in cellular adhesion has been reported as the monoclonal antibody 27E10 inhibited the attachment of monocytes to collagen and fibronectin. On the other hand, these extracellular matrix proteins induced the expression of calprotectin in parallel with the release of inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) and production of superoxide anions (Mahnke *et al.* 1995). The relationship between calprotectin expression and higher capacity to release TNFα has also been shown in human alveolar macrophages derived by bronchoalveolar lavage (Zheng *et al.* 1995). *In vitro* studies suggested an important role of calprotectin in extravasation of leukocytes by the attachment to endothelial cells *via* the MRP-14 subunit interacting mainly with endothelial heparan sulfate proteoglycans (Robinson *et al.* 2002). The molecules CD36 (Kerkhoff *et al.* 1998) and RAGE (receptor for advanced glycation end-products) (Hofmann *et al.* 2002) are two other putative receptors for calprotectin. The affinity of calprotectin for carboxylated glycans has also been demonstrated by another group (Srikrishna *et al.* 2001). Calprotectin binding to microvascular endothelial cells may also be induced by arachidonic acid (Eue and Sorg 2001). The signaling pathways of calprotectin are not fully elucidated, but involve MAP kinase cascade activation (Schaefer *et al.* 1999). The interaction of monocytic calprotectin with activated endothelium leads to its release (Frosch *et al.* 2000), which may account for the high calprotectin concentrations in the body fluids of patients with acute or chronic inflammatory diseases. Released calprotectin may be involved in inflammation by enhancing CD11b expression in human monocytes and by participating in the transendothelial migration mechanism (Hogg and Newton 1998).

Physiological role of soluble calprotectin

Calprotectin has antimicrobial and apoptosis-inducing activities, which are reversed by the addition of zinc. By sequestration of zinc, calprotectin inhibits MMPs (matrix metalloproteinases), zinc-dependent enzymes that are important in embryonic development, angiogenesis, wound healing, inflammation, cancer, and tissue destruction. In this way, calprotectin is capable of regulating many important processes in the body. Calprotectin also inhibits the microbial growth through competition for zinc. Zinc chelation that is mediated by histidine-rich regions of calprotectin represents an important antimicrobial mechanism in host defense (Clohessy and Golden 1995, Loomans *et al.* 1998). Calprotectin concentrations of 50-250 μg/ml were found to inhibit growth of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, lower concentrations (4-32 μg/ml) are sufficient to inhibit growth of *Candida albicans*. Cells expressing calprotectin are able to resist invasion by *Listeria monocytogenes* and *Salmonella enterica serovar Typhimurium* (Nisapakultorn *et al.* 2001). It is likely that calprotectin represents a defense mechanism by protecting neutrophils and other calprotectin-expressing cells against microbes that invade the host's cell cytoplasm.

Regulation of calprotectin synthesis

In addition to local regulation of calprotectin expression by proinflammatory cytokines, genetic factors might also be of vital importance. A previously non-described inborn error of zinc metabolism was identified by Sampson *et al.* (1997). These authors reported a child with hyperzincemia (> 200 μmol/l, reference range 11-18 μmol/l) associated paradoxically with symptoms of zinc deficiency (severe growth failure, hepatosplenomegaly, rashes, anemia, and impaired immune functions) due to zinc capturing of upregulated calprotectin (6.5 g/l, i.e. >1000 times normal). The fecal and urinary calprotectin content was within the normal range. It was suggested that this patient had a defect in the control of calprotectin synthesis or in calprotectin catabolism (more likely explanation). Hypercalprotectinemia led to zinc deficiency and generalized inflammatory disease. New patients with calprotectin dysregulation accompanied by recurrent infections, hepatosplenomegaly, anemia and systemic inflammation have recently been reported (Sampson *et al.*

2002), creating a new disease entity or syndrome.

Calprotectin as an inflammatory marker in clinical settings

The upregulation of serum calprotectin levels may occur in different immune and immunopathological reactions, especially in acute inflammation or Th1-mediated responses. In some respects, the sensitivity and dynamics of calprotectin seem to overcome traditional inflammatory markers such as C-reactive protein (CRP).

Organ transplantation

Our recent data showed a rapid increase in the serum levels of calprotectin in response to bacterial infection in kidney or heart allograft recipients but, also, during the course of acute rejection (Stříž *et al.* 2001a). Calprotectin may be a very sensitive marker of complications in organ transplantation, especially in combination with other inflammatory markers such as procalcitonin, extremely specific for bacterial systemic infections (Jarešová *et al.* 1999). Calprotectin usefulness in kidney allograft transplantation has also been confirmed by others (Burkhardt *et al.* 2001).

Pulmonary diseases

Calprotectin is a valuable marker at the very early stage of inflammatory reactions in human lungs (Stockley *et al.* 1984). It seems to be comparable to CRP in distinguishing between bacterial and viral infections. Plasma levels of 40 to 130 times the normal values were frequently seen during life-threatening infections such as septicemia, meningitis, or pneumonia (Sander *et al.* 1984). Patients with active tuberculosis had significantly increased plasma levels of calprotectin compared with pulmonary sarcoidosis and healthy controls. Human calprotectin increased *Mycobacterium tuberculosis* growth in a dose- and time-dependent manner (Pechkovsky *et al.* 2000).

Rheumatoid arthritis

It has been suggested that rheumatoid inflammation is mediated preferentially by activated pro-inflammatory Th1 cells (Hitchon and El-Gabalawy 2002). The concentrations of plasma calprotectin have been shown to be a convenient marker of disease activity and joint inflammation but not predictive of the outcome of patients with rheumatoid arthritis (Madland *et al.* 2002). In juvenile rheumatoid arthritis, calprotectin seems to be superior to conventional markers for monitoring pathological activity (Frosch *et al.* 2000).

Gut inflammation

Only a small proportion of patients with abdominal discomfort have organic disease, but a correct diagnosis can seldom be made by simple clinical examination. Additional diagnostic procedures must be employed, but these are expensive and involve a certain risk. Assessment of fecal calprotectin can be used as a screening test for selecting patients for further examination (Fagerhol 2000). The test can be performed on 1-2 g of random stool samples that are sent to the laboratory by regular mail, since the protein is remarkably stable in stools. This test has a high sensitivity and specificity for gastrointestinal cancers and IBD (inflammatory bowel disease). Fecal calprotectin levels reflect disease activity in IBD and can be used to monitor the response to treatment and detect relapses (Aadland and Fagerhol 2002). Upper gastrointestinal disorders showed a small difference in calprotectin levels compared to median calprotectin levels in normal adult subjects (4.5 mg/ml). Median fecal calprotectin was elevated significantly in esophageal and gastric carcinoma (30 mg/ml), colorectal carcinoma (53 mg/ml) and IBD (Crohn's disease, 31 mg/ml, ulcerative colitis, 116 mg/ml) (Summerton *et al.* 2002). Serum calprotectin discriminates well between active and inactive Crohn disease and may have considerable potential in the analysis of clinical disease activity in these patients (Lugering *et al.* 1995).

Cell expression of calprotectin in inflamed tissues

Respiratory system

In addition to serum or fecal values of calprotectin which can easily be determined by commercial ELISA kits, its local membrane expression provides another important piece of information. In the lungs, calprotectin can serve as a marker of freshly recruited, monocyte-like mononuclear phagocytes, expressed in 84 % of peripheral blood mononuclear cells but only in 10 % of alveolar macrophages of healthy human subjects (Stříž *et al.* 2001b). The percentage of calprotectin-positive macrophage correlates with the proportion of bronchoalveolar neutrophils (Stříž *et al.* 1993). A rapid influx of macrophages that expressed calprotectin was observed in fetal pig lungs a few hours

after the translocation of *Escherichia coli* from an experimentally infected amniotic cavity (Šplíchal *et al.* 2002). A similar influx was observed in young gnotobiotic piglets after the oral infection with *E. coli* and translocation of bacteria into the lungs. The ratio of lung cells containing calprotectin was higher after infection with the virulent O55 strain than after infection with non-pathogenic O86 strain that was also capable to translocate into the lungs of gnotobiotic piglets, and was much higher than the number of these cells in the lungs of germ-free animals (unpublished results). Not only in respiratory infections but also in lung transplantations do calprotectin-positive macrophages expand during acute rejection (Frachon *et al.* 1994).

Kidney

In kidney allograft transplantations, calprotectin-positive macrophages have been found to be an early acute cellular rejection marker together with increased parenchymal expression of adhesion molecules (Burkhardt *et al.* 1995, Goebeler *et al.* 1994). Calprotectin-positive macrophages may likewise play an important role in ANCA-positive renal vasculitis (Rastaldi *et al.* 2000). The expression of calprotectin in the kidney is not restricted only to mononuclear phagocytes (Rugtveit *et al.* 1996), but can also be detected in the tubular epithelium (Brandtzaeg *et al.* 1987) and collecting ducts (Helbert *et al.* 2001).

Skin

Calprotectin is strongly expressed in infiltrating inflammatory cells, but may also be involved in skin carcinogenesis (Gebhardt *et al.* 2002). Calprotectin can be found in almost all dermatoses associated with hyperproliferation of epithelial cells (Kelly *et al.* 1989) and during wound healing (Thorey *et al.* 2001). Following drug-induced epidermal necrolysis, calprotectin can be found in suprabasal layers and throughout the epidermis of bullous skin (Paquet and Pierard 2002). Calprotectin binding to the endothelium may also occur in the dermis. Calprotectin-positive macrophages were found to be associated with urticaria (Czarnetzki *et al.* 1990), contact dermatitis (Roth *et al.* 1992), or in local progression of melanoma (Brocker *et al.* 1988).

Oral cavity and bowels

Calprotectin is constitutively expressed in gingival keratinocytes. In periodontitis, higher levels are found in the gingival cervical fluid and tissue specimens. It confers resistance to infection by *Porphyromonas gingivalis* (Nisapakultorn *et al.* 2001). In Crohn's disease, a strong calprotectin immunoreactivity is present in epithelial cells adjacent to ulcerative and fissuring lesions in the bowels (Lugering *et al.* 1995).

Joints

Calprotectin is a marker present exclusively on infiltrating tissue macrophages but not on resident tissue macrophages; therefore, it is expressed in the rheumatoid arthritis synovial membrane by macrophages on the lining layer adjacent to the cartilage-pannus junction (Youssef *et al.* 1999).

Conclusions and prospects

Calprotectin represents a cytosolic antibacterial protein present in neutrophils, which may also be expressed on the membrane of monocytes and is involved in their recruitment to inflammation site by adhesive interactions with the endothelium. Upon neutrophil activation or monocyte adhesion to the endothelium, calprotectin is released and may provide not only bacteriostatic but also cytokine-like effects in the local environment. When calprotectin metabolism is affected at the systemic level, the zinc-binding properties of the protein may induce severe dysregulation of zinc homeostasis with severe clinical symptoms. In any case, there are several lines of evidence showing the importance of calprotectin in defense mechanisms and physiological functions of the immune system. The clinical usefulness of calprotectin as an inflammatory marker has been shown not only in gastroenterology, where determination of the protein in feces is a non-invasive parameter reflecting pathological processes going on in the mucosa. The serum level of calprotectin may be a very sensitive non-specific inflammatory marker in various clinical settings. On the other hand, our experience suggests the importance of being aware that in neutropenic patients the results may often be falsely negative. In this respect, the data should be either evaluated by monitoring the dynamics of serum calprotectin levels or in combination with other inflammatory markers.

The determination of calprotectin-positive monocytes/macrophages in a tissue is another clinically relevant issue and may be useful for assessing the influx of mononuclear phagocytes to affected tissue or organ.

On the other hand, the invasive nature of the biopsy procedure represents a limitation. Theoretically, a new area might emerge in the future in the field of recombinant calprotectin or calprotectin-like drug administration, but the pleiotropic effects of this protein should always be taken into account and a large body of evidence regarding calprotectin metabolism, signaling and function will have to be accumulated before starting such attempts.

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