MINIREVIEW

Calprotectin – a Pleiotropic Molecule in Acute and Chronic Inflammation

I. STŘÍŽ, I. TREBICHAVSKÝ

1Department of Immunology, Institute for Clinical and Experimental Medicine, Prague, 2Division of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Nový Hrádek, Czech Republic

Received February 25, 2003
Accepted August 8, 2003

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

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,
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

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>calprotectin, MRP8/14 protein, calgranulin, L1 protein, 27E10 antigen</th>
<th>(Bhardwaj et al. 1992; Brandtzaeg et al. 1987; Gebhardt et al. 2002; Roth et al. 2001)</th>
</tr>
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<tbody>
<tr>
<td>Molecular weight</td>
<td>36 kD</td>
<td>(Zwadlo et al. 1986)</td>
</tr>
<tr>
<td>Structure</td>
<td>24 kD heterodimer (or 48 kD tetramer) of 2 calcium-binding proteins MRP8 and MRP14 (S100A8 and S100A9)</td>
<td>(Itou et al. 2002; Roth et al. 1994; Strupat et al. 2000)</td>
</tr>
<tr>
<td>Structural relationship</td>
<td>S100 proteins family</td>
<td>(Kerkhoff et al. 1998; Kligman and Hilt 1988; Lewit-Bentley and Rety 2000)</td>
</tr>
<tr>
<td>Distribution</td>
<td>neutrophils, monocytes, acute phase macrophages, invariably in endothelial and epithelial cells</td>
<td>(Bhardwaj et al. 1992; Brandtzaeg et al. 1987; Doussiere et al. 2002; Helbert et al. 2001; Pillay et al. 1998; Roth et al. 1993)</td>
</tr>
<tr>
<td>Functions</td>
<td>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</td>
<td>(Clohessy and Golden 1995; Eue et al. 2002; Mahnke et al. 1995; Newton and Hogg 1998; Sampson et al. 2002)</td>
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</table>

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 (Vogantas 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 (Brandzaeg 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 (Srikrisna 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.
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.

**Acknowledgements**

This work was financially supported by grant No. ME 580 from the Ministry of Education, Youth and Sports of the Czech Republic and by grant No. 6843-3 from the IGA MZCR.

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**Reprint requests**
Assoc. Prof. Ilja Stříž, MD, PhD, Department of Immunology, Institute for Clinical and Experimental Medicine, Vedeňská 1958, 140 21 Prague 4, Czech Republic. E-mail: ilja.striz@medicon.cz