S100A12 provokes mast cell activation: A potential amplification pathway in asthma and innate immunity

Zheng Yang, PhD,[a]* Wei Xing Yan, PhD,[a]* Hong Cai, PhD,[a] Nicodemus Tedla, PhD,[a] Chris Armishaw, PhD,[b] Nick Di Girolamo, PhD,[a] Hong Wei Wang, PhD,[a] Taline Hampartzoumian, PhD,[a] Jodie L. Simpson, PhD,[c] Peter G. Gibson,[c] John Hunt, PhD,[a] Prue Hart, PhD,[d] J. Margaret Hughes, PhD,[e] Michael A. Perry, PhD,[a] Paul F. Alewood, PhD,[b] and Carolyn L. Geczy, PhD[a] *Sydney, Brisbane, New Lambton, and Perth, Australia*

Background: The calcium-binding protein S100A12 might provoke inflammation and monocyte recruitment through the receptor for advanced glycation end products.
Objective: Because inflammation elicited by S100A12 *in vivo* had characteristics of mast cell (MC) activation, we aimed to define the mechanism.
Methods: Various MC populations were used to test S100A12 activation assessed on the basis of morphology, histamine release, leukotriene production, and cytokine induction. MC dependence of S100A12-provoked inflammation was tested in mice and on the rat microcirculation by means of intravital microscopy. Immunohistochemistry localized S100A12 in the asthmatic lung, and levels in sputum from asthmatic patients were quantitated by means of ELISA. Expression of the receptor for advanced glycation end products was evaluated by means of RT-PCR and Western blotting.
Results: S100A12 provoked degranulation of mucosal and tissue MCs *in vitro* and *in vivo* and amplified IgE-mediated responses. It induced a cytokine profile indicating a role in innate/T_H1-mediated responses. S100A12-induced edema and leukocyte rolling, adhesion, and transmigration in the microcirculation were MC dependent. Eosinophils in airway tissue from asthmatic patients were S100A12 positive, and levels were increased in sputum. S100A12 responses were partially blocked by an antagonist to the receptor for advanced glycation end products, but MCs did not express mRNA or protein, suggesting an alternate receptor.
Conclusion: This novel pathway highlights the potential importance of S100A12 in allergic responses and in infectious and chronic inflammatory diseases.
Clinical implications: MC activation by S100A12 might exacerbate allergic inflammation and asthma. S100A12 might provide a novel marker for eosinophilic asthma. (J Allergy Clin Immunol 2007;119:106-14)

Key words: *S100A12, inflammation, mast cell activation, leukocyte migration, asthma, eosinophils, sputum, receptor for advanced glycation end products*

Mast cells (MCs) are critical to the pathogenesis of asthma, allergy, and parasitic infection; are initiators and effectors of innate immunity; and might contribute to the transition to acquired immunity.[1-4] MC activation could be particularly important in autoimmune diseases and modulated by inflammation,[1,2] but the activators in these settings are poorly understood. MCs are classically activated through antigen cross-linking of IgE bound to FcεRI. Ig-independent pathways include ligation of Toll-like receptors 2, 3, 4, 5, 7, and 9; complement components; some cytokines[1,2]; and some positively charged peptides.[5] Some stimulants upregulate various cytokine and chemokine genes without provoking degranulation.[1] The stored mediators released on degranulation increase blood vessel permeability and leukocyte extravasation, and newly synthesized mediators can also promulgate vascular changes that contribute to wound healing and chronic inflammation.[1-3]

There is evidence for communication between MCs and neutrophils in inflammatory responses, and neutrophil recruitment to sites of infection is MC dependent.[4,6] Histamine-releasing factors from diverse cell types, including activated mononuclear cells and neutrophils, are implicated.[5] S100A12 (also known as calgranulin C; extracellular newly identified RAGE-binding protein [EN-RAGE]) is a member of the S100 family of acidic calcium-binding proteins expressed in the human, but not in the mouse or rat genomes.[7] It comprises approximately 5% of neutrophil cytoplasmic protein and is induced in monocytes by LPS, TNF,[8] and IL-6.[9] S100A12 was proposed to affect MC-mediated responses by binding MC-stabilizing agents.[10]

S100A12 is a potent monocyte chemoattractant,[8,11] and S100A8 might be the functional ortholog of human

From [a]the School of Medical Sciences, The University of New South Wales, Sydney; [b]the Institute of Molecular Biosciences, The University of Queensland, Brisbane; [c]the Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, New Lambton; [d]the Telethon Institute for Child Health Research, University of Western Australia, Perth; and [e]the Respiratory Research Group, Faculty of Pharmacy, University of Sydney.
*These authors contributed equally to this article.
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Reprint requests: Carolyn L. Geczy, PhD, School of Medical Sciences, The University of New South Wales, Sydney, NSW 2052, Australia. E-mail: c.geczy@unsw.edu.au.
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Abbreviations used
BMMC: Murine bone marrow–derived mast cell
CBMC: Human cord blood–derived mast cell
EN-RAGE: Extracellular newly identified RAGE-binding protein
EP: Eosinophil peroxidase
LTC_4: Leukotriene C_4
MC: Mast cell
MCP: Monocyte chemoattractant protein
MIP: Macrophage inflammatory protein
PMC: Murine peritoneal mast cell
RAGE: Receptor for advanced glycosylation end products
WT: Wild-type

S100A12 in the mouse.[7] We showed that murine S100A8 (previously called CP-10, MRP8) generated a leukocyte infiltrate with composition and kinetics similar to delayed-type hypersensitivity responses to antigen.[12,13] S100A12 enhances neutrophil adhesion, and intravenous injection into mice stimulates neutrophil mobilization from bone marrow to inflammatory sites.[14] Bovine S100A12 injection caused edema, leukocyte recruitment, and upregulation of vascular cell adhesion molecule 1 on endothelium,[11] effects that were partially blocked by antagonists to the receptor for advanced glycosylation end products (RAGE), which is proposed as a pan-S100 protein receptor. RAGE interacts with diverse ligands implicated in inflammation, particularly in diabetes.[15] S100A12/RAGE ligation induces TNF and IL-1β in monocytes and adhesion receptors on endothelial cells through nuclear factor κB activation.[11,15] S100A12 is implicated in the pathogenesis of inflammatory bowel disease,[16] rheumatoid arthritis,[8,14,17] and vascular complications of diabetes[18] and plays a role in parasite defense.[19]

Here we show that S100A12 provoked MC degranulation and activation *in vitro* and potentiated MC responses to FcεRI cross-linking. S100A12 provoked leukocyte adhesion and extravasation in the microcirculation *in vivo*, and responses elicited *in vivo* were MC dependent. Importantly, eosinophils in lung lesions from patients with asthma expressed S100A12, and the protein level was significantly increased in sputum from patients with eosinophilc asthma compared with that seen in sputum from healthy subjects and subjects with other asthma subtypes. The S100A12-induced cytokine profile overlapped but was distinct from that induced by IgE cross-linking. The types and levels of chemokines produced indicate that S100A12 is a novel physiologic activator of MCs that might potentiate innate and allergic responses.

METHODS

Animals

Specific pathogen-free male Sprague-Dawley rats (6-8 weeks) and BALB/c female mice (6-10 weeks) were from the Biological Resource Centre, University of New South Wales. MC-deficient *Wf/Wf* mice and MC-replete mice C57Bl6/DBA(+/+; wild-type [WT]) were from Dr P. Hart. Experiments were performed according to ethical guidelines of the National Health and Medical Research Council of Australia.

S100A12 preparation

Recombinant and native S100A12 from human neutrophils were produced and purified as previously described.[8] Endotoxin content was less than 10 pg/10 μg of S100A12.

MC activation *in vitro*

Tissue culture media and reagents were filtered through Zetapor 0.2-μm membranes (Cuno, Meriden, Conn) to minimize endotoxin levels. Endotoxin levels were monitored with the chromogenic limulus amoebocyte lysate assay (Cape Cod Assoc, Wood Hole, Mass) and only used if levels were less than 10 pg/mL.

Human cord blood–derived MCs (CBMCs) were derived from human umbilical cord blood mononuclear cells, as previously described.[20] Nonadherent cells were transferred weekly for 9 to 10 weeks into media containing fresh cytokines. Maturity from 3 weeks was assessed weekly by means of tryptase, chymase, c-kit, and toluidine blue staining, and cells were used when more than 95% were metachromatic, c-kit high, and tryptase and chymase positive.

CBMCs (2×10^5/100 μL of RPMI/0.2% BSA) dispensed in duplicate into 96-well plates were incubated at 37°C for 1 hour with S100A12. For FcεRI cross-linking, CBMCs were primed with human myeloma IgE (2 μg/mL; Chemicon International, Temecula, Calif) for 5 days in the presence of IL-4 (10 ng/ml; Endogen, Rockford, Ill) and SCF (100 ng/mL; R&D Systems, Minneapolis, Minn), washed, and stimulated with or without rabbit anti-human IgE (1 μg/mL; ICN Pharmaceuticals, Costa Mesa, Calif) for 30 minutes, and histamine and leukotriene C_4 (LTC_4) levels were determined. For S100A12 potentiation, IgE-primed CBMCs were incubated with 0.01 to 10 μM of S100A12 for 1 hour before activation with a predetermined suboptimal dose of anti-IgE (0.07 μg/mL). A23187 (5 μmol/L) was used as the positive control. Histamine was measured by means of ELISA (Immuno-Biological Laboratories, Hamburg, Germany), and levels were calculated as above. LTC_4 levels determined by means of EIA (Cayman Chemicals, Ann Arbor, Mich) were expressed as nanograms per 10^6 cells. Means ± SD of duplicate assays from at least 3 donors are given.

IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1α, IFN-γ, TNF, granulocyte colony-stimulating factor, and GM-CSF in supernatants of CBMCs (2×10^5, n = 4) were measured with the Bio-plex system (Bio-Rad Laboratories, Hercules, Calif), and results were analyzed with Bio-plex Manager software. Sensitivity was 2.0 pg/mL for each cytokine (upper limit, 32 ng/mL).

Activation of human lung MCs

Samples of apparently normal lung tissue from patients (n = 2) undergoing surgery for lung cancer in the Central Area Health Service, Sydney, Australia, were obtained with consent under ethical guidelines. Duplicate samples of chopped parenchyma (50 mg each) in 0.5 mL of Krebs-Henseleit solution, pH 7.4, at 37°C were treated with S100A12 or 1 μM of A23187 for 45 or 90 minutes, placed on ice, and 0.3 mL mixed with 0.3 mL $HClO_4$. Histamine in supernatants and total histamine in 0.5-mL supernatant mixed with lung tissue/0.8 mol/L $HClO_4$ boiled for 15 minutes in 1.0 mL Krebs-Henseleit solution was measured by means of ELISA.

S100A12 levels in sputum

Subjects with airway disease (n = 21) attending the Department of Respiratory and Sleep Medicine, John Hunter Hospital (Newcastle),