Veterinary Immunology and Immunopathology 150 (2012) 47–52

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Serum amyloid A uptake by feline peripheral macrophages

Takashi Tamamoto, Koichi Ohno*, Yuko Goto-Koshino, Yasuhito Fujino, Hajime Tsujimoto

Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:
Received 1 May 2012
Received in revised form 2 August 2012
Accepted 8 August 2012

Keywords:
Serum amyloid A
Macrophage
Uptake
Feline
Dexamethasone

ABSTRACT

Serum amyloid A (SAA) is one of the major acute phase proteins in cats and humans. SAA concentrations increase in response to the inflammatory status and secondary amyloid A amyloidosis has been documented in cats. In order to control the SAA concentration, it is important to clarify how the SAA protein is metabolized. Although the details of SAA metabolism in the body remain unknown, human and murine research indicates that macrophages play a key role in SAA uptake. The objectives of this study were to demonstrate SAA uptake by feline macrophages and to evaluate the effects of lipopolysaccharide (LPS) and dexamethasone (Dex) on SAA uptake. The concentration of recombinant feline SAA added to a feline macrophage culture was decreased in a time-dependent manner and was significantly reduced after a 24-h incubation, as demonstrated by enzyme linked immunosorbent assay (ELISA). SAA uptake into feline peripheral macrophages was demonstrated by immunofluorescence microscopy. Pretreatment to macrophages with LPS did not affect this decrease in the SAA concentration, but this was significantly blocked by Dex pretreatment. In conclusion, SAA was incorporated by feline macrophages and pretreatment with Dex inhibited SAA uptake by macrophages in this study. Further investigation is needed to determine the molecules that influence SAA uptake by macrophages and the effect of clinical glucocorticoid usage on the SAA concentration in cats.

1. Introduction

Serum amyloid A (SAA) is one of the major acute phase proteins in many species, including humans (Kushner, 1988; Malle and de Beer, 1996) and cats (Kajikawa et al., 1999; Sasaki et al., 2003; Giordano et al., 2004). In the inflammatory state, these proteins are mainly synthesized in the liver due to the stimulation of inflammatory cytokines, such as IL-1, IL-6, and TNF-α. Depending on the extent of inflammation, serum SAA concentrations may increase up to a 1000-fold in humans (Kushner, 1988) and cats (Hansen et al., 2006; Tamamoto et al., 2008) compared with the levels in the non-inflammatory state. Thus, the SAA level is used as a marker of inflammation to assess the existence of inflammatory diseases (Nakayama et al., 1993; Hansen et al., 2006; Tamamoto et al., 2008) and the response to therapy (Tamamoto et al., 2009).

Although physiological roles of the SAA protein in the body are not fully understood, evidence suggests that SAA functions as an inflammatory mediator. For example, SAA can increase cytokine production in monocytes and macrophages (Hatanaka et al., 2007; Mullan et al., 2006; Song et al., 2009) and can stimulate nitric oxide production

Abbreviations: SAA, serum amyloid A; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; Dex, dexamethasone; IL, interleukin; TNF, tumor necrosis factor; AA, amyloid A; IFN, interferon; EDTA, ethylenediaminetetraacetic acid; PBMC, peripheral blood mononuclear cell; HBSS, Hanks' balanced salt solution; FCS, fetal calf serum; PMA, phorbol 12-myristate 13-acetate; rfSAA, recombinant feline serum amyloid A; IPTG, isopropyl beta-D-thiogalactosidase; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 0.1% Tween 20; Ig, immunoglobulin; IF, immunofluorescence; DAPI, 4′,6-diamidino-2-phenylindole; PI, propidium iodide; SR-B1, scavenger receptor class B type 1.

* Corresponding author. Tel.: +81 3 5841 5402; fax: +81 3 5841 5640.
E-mail address: aohno@mail.ecc.u-tokyo.ac.jp (K. Ohno).

0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2012.08.005

in macrophages (Sandri et al., 2008). Furthermore, SAA has a chemotactic effect on neutrophils and macrophages (Badolato et al., 1994; Su et al., 1999; Mullan et al., 2006; Hatanaka et al., 2007). This suggests that the SAA protein is produced as a result of inflammation and is an important factor in the inflammatory response.

SAA is a precursor of amyloid A (AA) fibrils, and sustained high SAA levels in the blood may give rise to secondary amyloidosis, which is known as reactive AA amyloidosis. The amyloid burden and mortality of human patients with AA amyloidosis correlated significantly with the serum SAA concentration (Lachmann et al., 2007). Furthermore, it was reported that amyloid fibril deposits regressed, and prognosis was improved in patients in whom SAA levels had been lowered to within the reference range by treatment (Gillmore et al., 2001). Therefore, controlling the SAA concentration is believed to be important in managing inflammatory responses and intercurrent diseases.

The SAA concentration has been shown to increase in many inflammatory diseases and tumors in cats (Hansen et al., 2006; Tamamoto et al., 2008). Although no report has shown a direct association between the SAA concentration and the development of AA amyloidosis in cats, causes of secondary AA amyloidosis have been reported in cats with chronic inflammatory diseases (van der Linde-Sipman et al., 1997). This suggests that controlling the SAA concentration is also clinically useful in cats with inflammation.

To control the SAA concentration, it is important to clarify how the SAA protein is metabolized. Previous in vitro studies in mice revealed that the SAA protein was incorporated into macrophages immediately after addition to culture medium (Rocken and Kisilevsky, 1998; Kinkley et al., 2006). Another study showed that the SAA protein disappeared in a time-dependent manner when it was added to a monocyte culture (Lavie et al., 1978). This occurs when SAA is cultured with monocytes but not with lymphocytes (Migita et al., 2001). Moreover, this phenomenon was inhibited by inflammatory stimuli, such as IL-1 and IFN-γ. These reports indicate that monocytes/macrophages play a key role in SAA metabolism and that this phenomenon is affected by various factors. However, no report has described SAA protein metabolism in cats.

The aims of this study were to demonstrate the uptake of the SAA protein by feline macrophages and to evaluate the difference in reactivity when the macrophages were pretreated with lipopolysaccharide (LPS) or dexamethasone (Dex).

2. Materials and methods

2.1. Cell preparation

Feline macrophages were prepared according to previously described protocols (Mizukoshi et al., 2009; Goto-Koshino et al., 2011). These procedures were conducted in accordance with the guidelines of the Animal Care Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo. Briefly, EDTA-treated whole blood from healthy cats was overlaid onto Ficoll-Paque Plus (GE Healthcare, Buckinghamshire, United Kingdom) and centrifuged at $800 \times g$ for 30 min at room temperature. Peripheral blood mononuclear cells (PBMCs) at the interface were resuspended in Hanks' balanced salt solution (HBSS; Sigma–Aldrich, St. Louis, MO, USA) and centrifuged at $200 \times g$ for 10 min to remove contaminating platelets. PBMCs were resuspended in RPMI 1640 (Sigma–Aldrich) supplemented with 10% fetal calf serum (FCS; Biowest, Nuaille, France), 100 U/mL penicillin, and 0.1 mg/mL streptomycin. The cells were plated on a 24-well tissue culture-treated plate (Corning, Lowell, MA, USA) at a concentration of 1×10^6 cells/well, stimulated with 10 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma–Aldrich), and cultured at 37 °C in 5% CO_2. Monocytes were allowed to attach to the plastic bottom of the plate for 24 h, and the plate was subsequently washed with culture medium to remove non-adherent cells. PMA-free culture medium (2 mL) was added to the culture plate, and the cells were cultured for 6 days to induce macrophage differentiation.

2.2. Recombinant feline SAA (rfSAA) production and purification

Recombinant feline SAA protein was produced as previously described (Tamamoto et al., 2008), with slight modifications. The plasmid vector into which the feline SAA gene was inserted was transformed in *Escherichia coli*, OverExpress C43 (DE3) Electrocompetent Cells (Lucigen, Middleton, WI, USA). The transformants were grown in Luria-Bertani medium, and expression of the recombinant protein was induced by culturing with 0.1 mM isopropyl beta-D-thiogalactosidase (IPTG; Sigma–Aldrich) for 2 h at 37 °C. Cultures were centrifuged at $10{,}000 \times g$ for 5 min at 4 °C, and the cell pellets were stored at −80 °C overnight. The expressed protein was purified using TALON Metal Affinity Resin (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. The protein concentration of the SAA fraction was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. ELISA for measurement of feline SAA

A rat anti-SAA monoclonal antibody and a rabbit anti-SAA polyclonal antibody were provided by Eiken Chemical Co., Ltd. (Tokyo, Japan). Each well of a 96-well plate (Corning) was coated with 2 μg of the anti-SAA polyclonal antibody by incubating overnight at 4 °C. After washing the wells with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST), 200 μL of 1% bovine serum albumin in PBST was added to each well and the plate was incubated at room temperature for 2 h to block non-specific binding. After washing the plate with PBST, 100 μL of diluted anti-SAA monoclonal antibody and 50 μL of diluted sample were added to each well. After a 1-h incubation period at 37 °C, the wells were washed with PBST and incubated with 100 μL horseradish peroxidase-conjugated anti-rat IgG at room temperature for 1 h. The plate was washed again and incubated with 50 μL of substrate solution (TMB; Nacalai Tesque, Kyoto, Japan) for 15 min. H_2SO_4 (2 N, 50 μL) was added to stop color development, and the absorbance was