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S100A8 Enhances IL-1 β Production from Nasal Epithelial Cells in Eosinophilic Chronic Rhinosinusitis

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Conflict of Interest

There are no conflicts of interest to declare.

Authors Contributions

YM and HI supervised the project. AN and TT analyzed the data and wrote the manuscript. SO, MH, YM, AN, and KT recruited and clinically characterized patients. DO and TK provided advice on project planning and data interpretation. All authors participated in discussion of the results and critically reviewed and approved the final draft.

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Abstract

Chronic rhinosinusitis is classified into eosinophilic chronic rhinosinusitis (ECRS) and non-eosinophilic chronic rhinosinusitis (NECRS). ECRS is a refractory allergic disease involving a variety of immune and epithelial cells. S100A8 is a damage-associated molecular pattern that is closely related to allergic inflammation. However, the pathological implications of S100A8 in ECRS have not been clarified. We evaluated the role of S100A8 in the pathogenesis of ECRS. Gene expression profiles of nasal polyps obtained from patients with ECRS or NECRS were evaluated using RNA sequencing. *S100A8* was identified as a significantly upregulated gene in nasal polyps associated with ECRS. Immunohistochemistry consistently revealed intense S100A8 staining in nasal polyps from patients with ECRS. Human nasal epithelial cells expressed the receptor for advanced glycation end products and Toll-like receptor 4. Recombinant S100A8 protein induced interleukin-1 β secretion in human nasal epithelial cells. Our data demonstrate that S100A8 results in production of interleukin-1 β in the nasal epithelium, which may be involved in the pathogenesis of ECRS.

INTRODUCTION

Chronic rhinosinusitis (CRS) is an airway disease with paranasal sinus inflammation. In East Asia, the phenotypes of CRS are heterogeneous, and CRS is subdivided in two groups according to Japanese clinical criteria: eosinophilic chronic rhinosinusitis (ECRS) and non-eosinophilic chronic rhinosinusitis (NECRS) ^{1 2 3}. ECRS is an allergic disease that causes olfactory disturbances, nasal obstruction, and nasal discharge. In addition, bronchial asthma is a common complication of ECRS, which is designated as an intractable disease in Japan. ECRS is a type 2 inflammatory disease involving various types of immune cells. Recent studies have shown that the most severe forms of eosinophilic inflammation, including ECRS, are caused by inflammation resulting from a combination of type 2 and type 3 cytokines ^{4 5}. The main treatments for ECRS are endoscopic sinus surgery (ESS) and systemic glucocorticoids. However, ESS has a high relapse rate, while long-term systemic glucocorticoid steroid therapy is associated with side effects. Therefore, molecularly targeted therapy based on an understanding of pathogenesis is an attractive alternative for treatment of ECRS.

Nasal epithelial cells are the interface between the host and the environment and act as the first defense mechanism. Nasal epithelial cells produce a variety of cytokines and matrix metalloproteinases that exacerbate allergic inflammation ^{6 7}. In particular, when cellular damage occurs, nasal epithelial cells release damage-associated molecular patterns (DAMPs). DAMPs contribute to the activation of various immune cells by type 2 and type 3 cytokines, and previous reports have suggested a relationship between DAMPs and allergic diseases. High mobility group box-1 (HMGB-1), a representative DAMP, enhances interleukin-33 production from nasal epithelial cells ⁸. However, the pathological implications of other DAMPs in CRS have not been sufficiently clarified.

S100 proteins are a group of small Ca²⁺-binding modulator proteins. Recent work has revealed that S100 proteins are involved in tumor metastasis and immune responses ⁹. In particular, S100A8 protein binds to Toll-like receptor 4 (TLR4) or the receptor for advanced glycation end products (RAGE) and initiates the activation of various immune cells and the production of reactive oxygen species and nitric oxide, contributing to the exacerbation of chronic inflammation ^{10 11 12 13}.

In this study, we attempted to discover the genes involved in the pathogenesis of ECRS via RNA sequencing. We found that *S100A8* gene expression is upregulated in

nasal polyps obtained from patients with ECRS. Thus, we sought to clarify the mechanism of ECRS by characterizing the effects of S100A8 on the nasal epithelium.

MATERIALS AND METHODS

Human subjects

Paranasal sinus tissue samples were collected from seven patients with chronic rhinosinusitis treated between 2014 and 2017 at the Department of Otorhinolaryngology Head and Neck Surgery, Osaka University Hospital. This study was approved by the ethics committee of Osaka University (No. 14463). Written informed consent was obtained from all subjects. CRS was defined according to the guidelines in the European Position Paper on Rhinosinusitis and Nasal Polyps¹⁴. Patients with ECRS were diagnosed according to criteria established by the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis².

RNA sequencing (n = 8 ECRS, n = 2 NECRS), reverse transcription quantitative polymerase chain reaction (RT-qPCR) (n = 8 ECRS, n = 4 NECRS), and immunohistochemistry (S100A8: n = 15 ECRS, n = 15 NECRS; RAGE, TLR4, IL1- β : n = 5 ECRS, n = 5 NECRS) were performed.

reverse transcription quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry experiments were performed three times.

RNA sequencing

Total RNA extracted from nasal polyps from two patients with ECRS and two patients with NECRS were evaluated using RNA sequencing. Library preparation was performed using the SMARTer® Stranded Total RNA Sample Prep Kit-Pico Input Mammalian (TaKaRa, Osaka, Japan). Sequencing was performed on an Illumina HiSeq 2500 platform in 75-base single-end mode. Illumina CASAVA 1.8.2 software was used for base calling. Sequenced reads were mapped to the human reference genome sequence (hg 19) using TopHat version 2.0.13 in combination with Bowtie2 version 2.2.3 and SAMtools version 0.1.19. The number of fragments per kilobase of exon per million mapped fragments (FPKMs) was calculated using Cufflinks version 2.2.1. The raw data have been deposited in the NCBI Gene Expression Omnibus database GSE172305).

RNA extraction and reverse transcription

RNA samples from tissue samples were extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA). Complementary DNA (cDNA) was synthesized using the ReverTra Ace qPCR RT Master Mix (TOYOBO, Osaka, Japan).

RT-qPCR

cDNA was analyzed by real-time RT-qPCR using GoTaq qPCR Master Mix (Promega, Madison, WI, USA) in ABI 7300 (Applied Biosystems, Waltham, MA, USA). Values were normalized to the expression of *Gapdh*, and the fold difference in expression relative to that of *Gapdh* is shown. The following primer sets were used: human S100A8 (forward: 5'-GGGATGACCTGAAGAAATTGCTA-3', reverse: 5'-TGTTGATATCCAACCTCTTTGAACCA-3'), human GAPDH (forward: 5'-CATGTTTCGTCATGGGGTGAACCA-3', reverse: 5'-AGTGATGGCATGGACTGTGGTCAT-3').

Immunohistochemistry

After deparaffinization of paraffin-fixed tissue, antigen retrieval was performed with autoclaving for 15 min at 125°C in an ethylenediaminetetraacetate buffer solution (pH 9). Endogenous peroxidase activity was blocked using REAL Peroxidase Blocking Reagent (Dako, Carpinteria, CA, USA). Sections were allowed to react overnight at 4°C with S100A8 antibody (1:2500; Novus Biologicals, Littleton, CO, USA), RAGE Antibody (1:2000; Sino Biological, Beijing, China), TLR4 antibody (1:4000; Santa Cruz Biotechnology, Dallas, TX, USA), or IL-1 β antibody (1:4000; Bioss Antibodies, Woburn, MA, USA). After incubation with Histofine Simple Stain MAX PO (R) (Nichirei, Tokyo, Japan), slides were developed using 3,3-diaminobenzidine as the chromogen.

Histological analysis

Positive cell numbers were assessed in three regions at random points with 500 high-power fields. The sum of the positive cell numbers was used as the score for each individual case.

Immunofluorescence

After deparaffinization of paraffin-fixed tissue, antigen retrieval was performed with autoclaving for 15 min at 125°C in an ethylenediaminetetraacetate buffer solution (pH 9). Sections were blocked with 1% BSA in PBS and were reacted with S100A8/MRP8 Antibody (1:100, LS-B1618-50; LS Bio, Seattle, WA, USA) or MBP Antibody (1:100, bs-0380R; Bioss Antibodies) at 4°C overnight. After incubation with Alexa Fluor 488 goat anti-rabbit IgG (cat A11008; Invitrogen) or Alexa Fluor 647 anti-mouse IgG2b (cat A21242; Invitrogen) for 3 h at room temperature, and 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA) was used for nuclear counterstaining.

Human primary cell culture

Human nasal epithelial cells (HNEpCs) (PromoCell, Heidelberg, Germany) were cultured at 37°C and 5% CO₂ using an airway epithelial cell growth medium with supplements (PromoCell).

Isolation of eosinophils, neutrophils or peripheral blood mononuclear cells (PBMCs)

Eosinophils from healthy peripheral blood were isolated by negative selection using a MACS Isolation Kit (Miltenyi Biotec, Tokyo, Japan). Neutrophils and PBMCs were isolated with the Polymorphprep™ protocol (Abbott Diagnostics Technologies AS, Oslo, Norway).

Western blot

HNEpCs were lysed with buffer containing protease inhibitor. Eosinophils, neutrophils, and PBMCs were isolated from the peripheral blood of a healthy individual and lysed with buffer containing protease inhibitor. TLR4, RAGE, and S100A8 in the cell lysates were detected by western blotting. For electrophoresis, lysates were boiled for 5 min in sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis sample buffer containing 0.125 mM Tris-HCl (pH 6.8), 20% glycerol, 4% SDS, and 10% 2-mercaptoethanol, and loaded onto Next Page GLX-1F5B gels (Gellex International, Tokyo, Japan). Proteins were transferred to membranes using the Trans-Blot Turbo system (Bio-Rad, Hercules, CA, USA). Membranes were blocked for 1 h at room

temperature in Blocking-One buffer (Nacalai Tesque, Tokyo, Japan). After blocking, membranes were incubated with anti-RAGE antibody (#42544, 1:1000; Cell Signaling Technology Japan, Tokyo, Japan), TLR4 antibody (#sc-293072, 1:200; Santa Cruz Biotechnology) or S100A8/MRP8 Antibody (#LS-B1618-50, 1:100; LS Bio, Seattle, WA, USA) at 4°C overnight, and reacted with anti-rabbit IgG antibody or anti-mouse IgG antibody for 1 h at room temperature. The signal was then developed with Chemi-Lumi One (Nacalai Tesque).

Human nasal epithelial cells

HNEpCs were treated for 24 h with recombinant S100A1 (10 µg/ml) (R&D Systems, Minneapolis, MN, USA), recombinant S100A3 (10 µg/ml) (Novus Biologicals, Littleton, CO, USA), or recombinant S100A9 (10 µg/ml) (R&D Systems). Human recombinant S100A8 (R&D Systems) was used for stimulation at a final concentration of 1 µg/ml or 10 µg/ml. Supernatants were collected and preserved at -80°C.

Enzyme-linked immunosorbent assay (ELISA) and flow cytometric bead array

Cytokine levels in HNEpC culture supernatants were measured with a Cytometric Bead Array Human IL-1β Flex Set (BD Bioscience, Tokyo, Japan) or Human TNF-α DuoSet ELISA (R&D Systems).

All experiments were performed in accordance with relevant guidelines and regulations.

Statistical analysis

All statistical analyses were performed with Prism version 7 (GraphPad Software, San Diego, CA, USA). Data were expressed as means ± SD. Comparisons between two groups were performed using Student's t-test, and $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Upregulated genes in nasal polyps from patients with ECRS

In order to investigate the factors involved in the pathogenesis of ECRS, we first performed RNA sequence analysis of nasal polyps obtained from patients with ECRS or NECRS. *S100A8* was identified as a significantly upregulated gene in nasal polyps associated with ECRS (Table 1). The expression of other S100 protein-related genes is

shown in Table 2. *S100A9* was significantly upregulated in nasal polyps from patients with ECRS, while *S100A1* and *S100B* were significantly upregulated in nasal polyps from patients with NECRS. Therefore, we hypothesized that *S100A8* plays an important role in the pathogenesis of ECRS. We evaluated the expression of S100A8 in locally inflamed tissue of patients with ECRS.

Evaluation of S100A8 expression in nasal polyps associated with ECRS

First, we confirmed that the relative expression of S100A8 measured by RT-qPCR was slightly higher in ECRS than in NECRS, but not significantly (Figure 1A). In immunohistochemistry studies, anti-S100A8 antibody staining of eosinophils that had infiltrated nasal polyps was stronger in patients with ECRS than in those with NECRS (Figure 1B–D). The number of cells stained with S100A8 was significantly higher in ECRS than in NECRS (Figure 1E, F). These results indicate that the increase in S100A8 in nasal polyps associated with ECRS may be due to infiltrating eosinophils. We also confirmed the expression of S100A8 in peripheral blood eosinophils, neutrophils, and mononuclear cells by western blotting (Supplemental Figure 1).

Expression of the receptor for S100A8 in human nasal epithelial cells

To further investigate how S100A8 functionally contributes to ECRS, we first confirmed the expression of TLR4 and RAGE in HNEpCs using western blotting. Both TLR4 and RAGE were expressed in HNEpCs in the steady state (Figure 2A). Next, the expression of TLR4 and RAGE were compared by immunohistochemistry in ECRS and NECRS. Interestingly, the expression of TLR4 was significantly higher in ECRS than in NECRS (Figure 2B). The expression of RAGE was nonsignificantly higher in ECRS than NECRS (Figure 2C).

S100A8 protein pathophysiology in nasal epithelial cells

Previous reports suggested that various cells promote proinflammatory cytokine production via S100A8 stimulation. Thus, we examined cytokine production by nasal epithelial cells following stimulation with recombinant S100A8 protein for 24 or 48 h. Interestingly, S100A8 induced interleukin-1 beta (IL-1 β) production in nasal epithelial cells (Figure 3A) in a dose-dependent manner (Figure 3B). However, stimulation by S100A8 did not induce interferon-gamma production (Figure 3C). Next, we examined cytokine production by nasal epithelial cells following stimulation with recombinant S100A1, S100A9, and S100A3 proteins as negative controls. S100A8 induced IL-1 β

production in nasal epithelial cells more strongly than S100A1 or S100A3. S100A9 induced IL-1 β production as strongly as S100A8 (Figure 3D). In immunohistochemistry, IL-1 β was more highly expressed in ECRS than in NECRS, but not significantly (Supplemental Figure 2).

DISCUSSION

In this study, we demonstrated the clinical implications of S100A8 in ECRS, a refractory disease involving type 2 inflammation. Some previous reports suggested that DAMPs released by damaged tissue might contribute to the exacerbation of allergic diseases⁸. S100A8, a type of DAMP, is produced mainly by blood cells^{15 16 17}. It has been reported that RAGE and TLR4 are receptors for S100A8, and S100A8 induces inflammation by binding to these receptors. Thus, S100A8 is closely related to allergic dermatitis and autoimmune diseases, but its role in ECRS has not been fully explored^{18 19}.

RNA sequencing showed that *S100A8* was more highly expressed in nasal polyps associated with ECRS than those associated with NECRS. Among other genes in the S100 protein family, *S100A9* was also significantly upregulated in nasal polyps associated with ECRS. Previous studies used RNA sequencing to compare gene expression in nasal polyps associated with ECRS and NECRS²⁰. In RT-qPCR in this study, there was no significant difference in *S100A8* expression between ECRS and NECRS, but expression was nonsignificantly higher in ECRS. However, we believe that the lack of significant difference between ECRS and NECRS is due to the small number of cases examined by RT-qPCR.

However, there are no studies that evaluated the expression of S100 family proteins in local tissues from patients with ECRS. It has been reported that S100A8 is mainly secreted by neutrophils and monocytes^{15 16 17}. Thus, increased S100A8 gene expression in nasal polyps associated with ECRS might be due to the infiltration of various immune cells. In this study, histological evaluation revealed S100A8 protein expression in tissue-infiltrating eosinophils. No previous reports have described the contribution of eosinophils to the production of S100A8; this finding is new. Further studies will be needed to elucidate the factors that induce the production of S100A8 in eosinophils.

We evaluated the role of S100A8 in the exacerbation of ECRS. Nasal epithelial cells were predicted as a target of S100A8 action. Therefore, we assessed the receptors

for S100A8 in the nasal epithelium. Previous reports have shown the expression of RAGE and TLR4 in lower airway epithelial cells^{18 19}. This study showed that both receptors are expressed in the upper airway as well. In addition, S100A8 has been reported to contribute to the production of various proinflammatory cytokines and increased production of MUC5AC¹¹. Hence, we stimulated nasal epithelial cells with S100A8 and found increased production of IL-1 β , an important factor for both type 2 and type 3 inflammation. These results are consistent with recent studies showing that severe eosinophilic inflammation is caused by inflammation mediated both by type 2 and type 3 cytokines^{4 5 21 22 23}.

We also showed that the expression of TLR4 was higher in ECRS than in NECRS. This result suggests that S100A8 may be highly expressed in ECRS due to the higher expression of TLR4 in ECRS.

This study has some limitations. We did not investigate whether IL-1 β production by nasal epithelial cells is mediated by TLR4 or RAGE receptors. Additionally, in immunohistochemistry, the expression of IL-1 β was nonsignificantly higher in ECRS than NECRS.

However, the study has three novel features. Comprehensive analysis revealed that *S100A8* is upregulated in nasal tissues of patients with ECRS. S100A8 protein expression was upregulated in eosinophils infiltrating nasal polyps in ECRS and enhances IL-1 β production in upper airway epithelial cells. S100A8 worsens allergic inflammation, implying that S100A8 blockade could promote recovery from ECRS. Therefore, studies with mouse models, as well as clinical tests of S100A8-blocking therapies, should be pursued in the future.

In summary, we found increased expression of *S100A8* gene expression in nasal polyps from patients with ECRS. S100A8 might be derived from eosinophils that have infiltrated nasal tissue. We also demonstrated that S100A8 enhances IL-1 β production in nasal epithelial cells, which may be involved in the pathogenesis of ECRS.

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Figure legend

Figure 1 S100A8 was expressed in eosinophils that have infiltrated nasal tissue.

A. Gene levels of S100A8 were measured by RT-qPCR and compared (n = 8 ECRS, n = 4 NECRS).

B, C. Hematoxylin and eosin staining of nasal tissues and immunohistochemical detection of S100A8. Images are of representative samples from patients with ECRS. Scale bars, 100 μ m.

D. Immunofluorescence staining for S100A8 (green), MBP (red), or DAPI (blue). Scale bars, 100 μ m.

E. Expression of S100A8 in nasal polyps from patients with ECRS or NECRS.

F. Immunohistochemical staining for S100A8; positive cells were counted (n = 15 ECRS, n = 15 NECRS).

NS, not significant, *P < 0.05, Student's t-test. (A), (F). Values are means \pm SD. MBP, major basic protein; DAPI, 4',6-diamidino-2-phenylindole

Figure 2. Expression of S100A8 receptor on HNEpCs.

A. Western blots showing that both TLR4 and RAGE are expressed in HNEpCs. Full-length blots/gels are presented in Supplementary Figure 3.

B. Immunohistochemical staining for TLR4; positive cells were counted (n = 5 ECRS, n = 5 NECRS). Left: representative images for ECRS and NECRS; right: number of positive cells.

C. Immunohistochemical staining for RAGE; positive cells were counted (n = 5 ECRS, n = 5 NECRS). Left: representative images for ECRS and NECRS; right: number of positive cells.

NS, not significant. *P < 0.05, Student's t-test (B), (C). Values are means \pm SD.

TLR4, Toll-like receptor 4; RAGE, receptor for advanced glycation end products; HNEpCs, human nasal epithelial cells.

Figure 3. IL-1 β production by HNEpCs stimulated with recombinant S100A8 protein.

- A. HNEpCs were stimulated with recombinant S100A8 protein (10 μ g/ml) with control for 24 or 48 h. IL-1 β levels in culture supernatant were determined using CBA.
- B. HNEpCs were stimulated with recombinant S100A8 protein (1 μ g/ml or 10 μ g/ml) with control for 24 h. IL-1 β levels in culture supernatant were determined using CBA.
- C. HNEpCs were stimulated with recombinant S100A8 protein (10 μ g/ml) with control for 24 h. INF- γ levels in culture supernatant were determined using ELISA.
- D. HNEpCs were stimulated with recombinant S100A1 protein (10 μ g/ml), recombinant S100A3 protein (10 μ g/ml), recombinant S100A8 protein (10 μ g/ml), or recombinant S100A9 protein (10 μ g/ml) with control for 24 h. IL-1 β levels in culture supernatant were determined using CBA.

NS, not significant, **P < 0.01, ***P < 0.005 Student's t-test (A), (B), (C), (D). Values are means \pm SD.

HNEpCs, human nasal epithelial cells; IL-1 β , interleukin-1 beta; CBA, cytometric bead array; Ctrl, control.

Table 1. Gene expression profile of nasal polyps from patients with eosinophilic chronic rhinosinusitis (ECRS) versus non-eosinophilic chronic rhinosinusitis (NECRS).

mRNA	Fold change	P
<i>CLC</i>	470.1	0.001
<i>TNFAIP8L2-SCNMI</i>	380.5	0.013
<i>S100A8</i>	313.0	0.047

Table 2. S100 family protein gene expression in nasal polyps from patients with eosinophilic chronic rhinosinusitis (ECRS) versus non-eosinophilic chronic rhinosinusitis (NECRS).

mRNA	Fold change	P
<i>S100A1</i>	4.357	0.030
<i>S100A2</i>	-10.813	0.472
<i>S100A3</i>	-1.489	0.885
<i>S100A4</i>	-1.238	0.710
<i>S100A5</i>	-2.106	0.811
<i>S100A6</i>	-1.824	0.236
<i>S100A7</i>	3.924	0.423
<i>S100A7A</i>	-3.347	0.196
<i>S100A7L2</i>	1.000	Not a number
<i>S100A8</i>	-313.013	0.047
<i>S100A9</i>	-13.014	0.031
<i>S100A10</i>	-1.637	0.112
<i>S100A11</i>	-2.158	0.051
<i>S100A12</i>	-3.444	0.708
<i>S100A13</i>	-2.122	0.223
<i>S100A14</i>	1.195	0.654
<i>S100A16</i>	2.314	0.125

<i>S100B</i>	111.508	0.010
<i>S100G</i>	1.000	Not a number
<i>S100P</i>	-4.583	0.100
<i>S100PBP</i>	1.395	0.733
<i>S100Z</i>	-17.283	0.059

Figures



Figure 1

S100A8 was expressed in eosinophils that have infiltrated nasal tissue. A. Gene levels of S100A8 were measured by RT-qPCR and compared (n = 8 ECRS, n = 4 NECRS). B, C. Hematoxylin and eosin staining of nasal tissues and immunohistochemical detection of S100A8. Images are of representative samples from patients with ECRS. Scale bars, 100 μ m. D. Immunofluorescence staining for S100A8 (green), MBP (red), or DAPI (blue). Scale bars, 100 μ m. E. Expression of S100A8 in nasal polyps from patients with ECRS or NECRS. F. Immunohistochemical staining for S100A8; positive cells were counted (n = 15 ECRS, n = 15 NECRS). NS, not significant, *P < 0.05, Student's t-test. (A), (F). Values are means \pm SD. MBP, major basic protein; DAPI, 4',6-diamidino-2-phenylindole



Figure 2

Expression of S100A8 receptor on HNEpCs. A. Western blots showing that both TLR4 and RAGE are expressed in HNEpCs. Full length blots/gels are presented in Supplementary Figure 3. B. Immunohistochemical staining for TLR4; positive cells were counted (n = 5 ECRS, n = 5 NECRS). Left: representative images for ECRS and NECRS; right: number of positive cells. C. Immunohistochemical staining for RAGE; positive cells were counted (n = 5 ECRS, n = 5 NECRS). Left: representative images for ECRS and NECRS; right: number of positive cells. NS, not significant. *P < 0.05, Student's t-test (B), (C). Values are means \pm SD. TLR4, Toll-like receptor 4; RAGE, receptor for advanced glycation end products; HNEpCs, human nasal epithelial cells.



Figure 3

IL-1 β production by HNEpCs stimulated with recombinant S100A8 protein. A. HNEpCs were stimulated with recombinant S100A8 protein (10 μ g/ml) with control for 24 or 48 h. IL-1 β levels in culture supernatant were determined using CBA. B. HNEpCs were stimulated with recombinant S100A8 protein (1 μ g/ml or 10 μ g/ml) with control for 24 h. IL-1 β levels in culture supernatant were determined using CBA. C. HNEpCs were stimulated with recombinant S100A8 protein (10 μ g/ml) with control for 24 h. INF- γ levels in culture supernatant were determined using ELISA. D. HNEpCs were stimulated with recombinant S100A1 protein (10 μ g/ml), recombinant S100A3 protein (10 μ g/ml), recombinant S100A8 protein (10 μ g/ml), or recombinant S100A9 protein (10 μ g/ml) with control for 24 h. IL-1 β levels in culture supernatant were determined using CBA. NS, not significant, **P < 0.01, ***P < 0.005 Student's t-test (A), (B), (C), (D). Values are means \pm SD. HNEpCs, human nasal epithelial cells; IL-1 β , interleukin-1 beta; CBA, cytometric bead array; Ctrl, control.

Supplementary Files

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