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Modulation of CD93 molecule in a human monocyte-like cell line (U937) treated with nickel

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Abstract

In this study, we demonstrated the modulation of CD93 molecules (both membrane-bound and soluble-form) in a human monocyte-like cell line, U937 treated with nickel (Ni^{2+}) to model contact hypersensitivity (CHS), such as metal allergy. Ni^{2+} induced the apoptosis of the U937 cells accompanied by the enhanced secretion of an inflammatory cytokine, interleukin-8 (IL-8). The percentages and mean fluorescence intensities (MFIs) of membrane-bound CD93 (mCD93) expressed on U937 cells were significantly decreased after treatment with Ni^{2+} when examined using flow cytometry with a CD93 monoclonal antibody (mAb) (mNI-11), which was established in our laboratories. In contrast, the secretion of soluble-form CD93 (sCD93) from U937 cells increased markedly after treatment with Ni^{2+}. Taken together, these findings suggest that the modulation of CD93 molecules (both mCD93 and sCD93) might serve as a new biomarker for analyzing the inflammatory reaction in CHS induced by Ni^{2+}.

Key words : CD93 molecule, contact hypersensitivity, nickel, U937 cells.

Introduction

Nickel is a ubiquitous metal used in a wide variety of products for health care, household use, and architecture. On the other hand, nickel can cause metal allergy because of the transfer of nickel ions under aqueous conditions [1]. Allergies to nickel ion (Ni^{2+}) are the most frequent cause of contact hypersensitivity (CHS) in industrialized countries [2]. Sensitization to nickel can occur at any age, even in newborns, indicating that the immune system in children can present an appropriate response to contact allergens from an early age, though sensitization might occur less often than in adults [3-5].

Human CD93 has a molecular weight (m.w.) of about 90-100 kDa and is a heavily *O*-glycosylated type I transmembrane protein consisting of unique C-type lectin domains [6]. CD93 is selectively expressed on myeloid cell lineages (monocytes and granulocytes) and endothelial cells and is involved in innate and adaptive immune responses [7]. Biologically, CD93 reportedly plays an important role in the exclusion/clearance of apoptotic/damaged cells in various immune responses [8].

The modulation of membrane-bound CD93 (mCD93) expression has been investigated in various types of cells. We previously reported that a protein kinase C activator (PKC), phorbol myristate acetate (PMA), effectively up-regulated mCD93 expression in several cultured cell lines [9].

Meanwhile, CD93 is reportedly shed/secreted from the surface of activated human monocytes and granulocytes (neutrophils) *in vitro*, resulting in the shedding/secretion of a soluble-form of CD93 (sCD93) [10]. In particular, sCD93 shedding was detected *in vitro* in response to inflammatory mediators, such as tumor-necrosis factor (TNF)-

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α and interleukin-8 (IL-8) (which are related to allergy development), endotoxin lipopolysaccharide (LPS), and PMA [10-12]. Therefore, we can easily hypothesize that the modulation of mCD93 and sCD93 is closely associated with various diseases involving inflammation, including allergy.

In the present study, to analyze the roles of the CD93 molecule in allergy with inflammation induced by Ni^{2+}, we established a new CD93 monoclonal antibody (mAb) (mNI-11) [6, 13] and demonstrated the modulation of mCD93 and sCD93 in a human monocyte-like cell line (U937) treated with Ni^{2+}, using the above-mentioned mAb as a probe for the analysis.

Materials and Methods

Cell line

The human monocyte-like cell line (U937) used in the present study was supplied by the Health Science Research Resource Bank (HSRRB) (Tokyo, Japan). U937 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with 10 mM HEPES buffer, 2 mM glutamine and 10% fetal calf serum (FCS) (referred to as complete medium).

Antibodies and reagents

Nickel ($NiSO_4$ $6H_2O$) was purchased from Nakarai Tesque Co. (Tokyo, Japan) and dissolved in distilled water at a concentration of 10 mM (Ni^{2+}). Horseradish peroxidase (HRPOD)-conjugated streptavidin was purchased from Cosmo Bio Co. (Tokyo, Japan). Phycoerythrin (PE)-conjugated and non-conjugated CD93 monoclonal antibodies (mAbs) (mNI-11; mouse IgG1) [6, 13] were established in our laboratories. PE-conjugated isotype-matched normal mouse IgG1 was purchased from MBL Co. (Nagoya, Japan). A biotin-conjugated CD93 mAb (X-2; mouse IgG1) was purchased from Cosmo Bio Co. (Tokyo, Japan). Recombinant CD93 protein (TP306980) was purchased from OriGene Technologies, Inc. (USA).

Preparation of U937 cells treated with Ni^{2+}

U937 cells ($1x10^5$) in complete medium were seeded in 96-well plates (Sumitomo Co., Tokyo) and treated with or without various concentrations of Ni^{2+} for 16 hrs in a CO_2 incubator. The treated cells were harvested, and the modulation of membrane-bound CD93 (mCD93) expression was analyzed using flow cytometry with a PE-conjugated CD93 mAb (mNI-11). Culture supernatants were used to detect soluble-form CD93 (sCD93) or interleukin-8 (IL-8) using an enzyme-linked immunoassay (EIA) system, respectively.

Detection of apoptotic cells

The enrichment of mono- and oligonucleosomes in the cytoplasm of apoptotic cells suggests that DNA degradation occurs before plasma membrane breakdown. Thus, we performed apoptotic monitoring of U937 cells treated with or without various concentrations of Ni^{2+} by determining the presence of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates using an apoptosis detection EIA kit (Roche Applied Science). These experiments were repeated five times.

Flow cytometry

U937 cells ($1x10^5$) treated with or without various concentrations of Ni^{2+} for 16 hrs were washed in cold phosphate-buffered saline (PBS) containing 0.1% NaN_3 (referred to as washing buffer), and incubated in PBS containing 25% normal goat serum, 1 mg/mL normal human IgG and 0.1% NaN_3 to block the Fc receptor (FcR) for IgG for 10 min on ice. After washing three times, the cells were incubated with an optimal concentration of PE-conjugated CD93 mAb (mNI-11) for 30 min at room temperature. Isotype-matched PE-conjugated normal mouse IgG1 was used as a negative control. After washing twice in the washing buffer, the cells were re-suspension in PBS containing 2% FCS and 0.1% NaN_3. The percentages and mean fluorescence

intensities (MFIs) of positively stained cells for the CD93 mAb (mNI-11) were determined by the use of the FACScan system (Becton Dickinson Co.). The experiment was repeated three times.

Assay for soluble-form CD93 (sCD93) in culture supernatants

Enzyme immunoassay (EIA) plates (Sumitomo Co., Tokyo) were coated with 500 ng/mL of CD93 mAb (mNI-11) for 18 hrs in carbonate biocarbonate buffer (0.01M $NaCO_3$, 0.035M $NaHCO_3$, pH9.6) at 4℃. The wells were then washed five times with PBS containing 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 2% BSA for 60 min at room temperature. Culture supernatant (50 μL) from U937 cells treated with or without various concentrations of Ni^{2+} for 16 hrs was then added to each well. A purified recombinant protein of CD93 (TP306980) was added to each well as a positive or standard control. Thereafter, 50 μL of biotin-conjugated CD93 mAb (X-2) (x5000 dilution with PBS-T containing 0.1% BSA) was added to each well, followed by incubation for 60 min at room temperature with shaking. The wells were washed eight times with PBS-T, and 100 μL of HRPOD-conjugated streptavidin (x5000 dilution with PBS-T containing 0.1% BSA) was added to each well and incubated for 30 min at room temperature with shaking. The wells were washed nine times with PBS-T, and 100 μL of the substrate-chromogen (TMB) (Cosmo Bio Co.) was added to each well, followed by 5-10 min of incubation at room temperature. The reaction was stopped by the addition of 0.5-N HCl, and the optical density (O.D.) was read at 450 nm using a multichannel EIA microplate reader (TOHSO Co., Tokyo). The experiment was repeated five times.

Assay for interleukin-8 (IL-8) in culture supernatants

The detection of IL-8 in culture supernatants from U937 cells treated with or without various concentrations of Ni^{2+} for 16 hrs was performed using an IL-8 detection EIA kit (MBL Co.). The experiments were repeated three times.

Results

Induction of apoptosis in U937 cells treated with Ni^{2+}

We performed apoptotic monitoring of the U937 cells treated with or without various concentrations of Ni^{2+} using an apoptosis detection EIA kit. Figure 1 shows that apoptosis was strongly induced in U937 cells treated with Ni^{2+} in a dose-dependent manner.

Enhancement of IL-8 secretion from U937 cells treated with Ni^{2+}

IL-8 secretion from U937 cells treated with or without various concentrations of Ni^{2+} was evaluated using an EIA kit. As shown in Fig. 2, treatment with Ni^{2+} significantly ($P < 0.05$) enhanced IL-8 secretion from U937 cells into the culture supernatants.

Decrease in membrane-bound CD93 (mCD93) expression on U937 cells treated with Ni^{2+}

mCD93 expression on U937 cells treated with or without various concentrations of Ni^{2+} was analyzed using a CD93 mAb (mNI-11) and a flow cytometry analysis. Figure 3 shows that both the percentages and the mean fluorescence intensities (MFIs) of mCD93 expression on U937 cells decreased significantly after treatment with Ni^{2+} ($P < 0.05$). Figure 4 shows the typical flow cytometric profiles of mCD93 expression patterns in U937 cells treated with or without Ni^{2+}.

Increase in soluble-form CD93 (sCD93) secretion from U937 cells treated with Ni^{2+}

sCD93 secretion from U937 cells treated with

or without various concentrations of Ni^{2+} was demonstrated using an EIA system originally constructed in our laboratories. As shown in Fig. 5, Ni^{2+} treatment significantly ($P < 0.05$) increased sCD93 secretion from U937 cells into the culture supernatants in a dose-dependent manner.

Discussion

In this study, we found that mCD93 expression on U937 cells treated with Ni^{2+} decreased significantly, while sCD93 secretion from this same cell line under the same conditions increased significantly accompanied by the enhancement of IL-8 secretion during the apoptotic process.

We can suggest some possible reasons for the decrease of mCD93 on U937 cells treated with Ni^{2+}. One possible reason for the decrease of mCD93 expression is the shedding/release of this molecule from the cell membrane after treatment with Ni^{2+}. In fact, the amount of secreted sCD93 in the culture supernatants from U937 cells increased significantly after treatment with Ni^{2+}. Another reason for the decrease in mCD93 expression on U937 cells treated with Ni^{2+} might be the suppression of the phosphorylation (unphosphorylation) of some proteins via the action of protein kinases (PKCs).

In the modulation of mCD93, we previously reported that a PKC activator, phorbol myristate acetate (PMA), effectively up-regulated mCD93 expression on several cultured cell lines, including U937 cells, through the phosphorylation of some protein(s) by PKC delta isoenzyme [9]. Furthermore, since the glycosylation of the CD93 molecule is an important factor for the stability of cell surface expression [14], the decrease in mCD93 expression on U937 cells treated with Ni^{2+} may depend on unglycosylation through the suppression of phosphorylation.

Moreover, we previously reported that the expression of mCD93 is specifically decreased by various apoptosis-induced chemical substances [15] and anti-CD95 (anti-Fas) mAb (CH-11) [16]. Thus, the decrease of mCD93 expression on U937 cells treated with Ni^{2+} may be associated with some intracellular apoptotic signals. In fact, U937 cells treated with Ni^{2+} underwent apoptosis, as indicated by the DNA degradation that was identified by the production of mono- and oligonucleosomes in the cytoplasmic fraction.

On the other hand, mCD93 is reportedly shed from activated human myeloid cells (monocytes and neutrophils) as the shedding/secretion of sCD93 [10]. In particular, sCD93 shedding/secretion was detected *in vitro* in response to treatment with LPS, TNF-α, IL-8 and PMA [10-12]. Therefore, we can easily suggest that the modulation of sCD93 secretion is tightly associated with inflammatory responses. In fact, several studies have reported that sCD93 shedding/secretion is closely related to some diseases with inflammation in humans and mice [11, 17].

Recently, toll-like receptor (TLR)-4, T cell receptors (TCRs), and some co-stimulatory CD molecules were reported to play crucial roles in the inducement/development of contact hypersensitivity (CHS) in response to Ni^{2+} [18, 19]. Thus, cross-talk signal(s) through TLR, TCRs, and co-stimulatory CD molecules, such as the CD93 molecule, in response to Ni^{2+} might be involved in the development of allergy with inflammation. Although the detailed reasons for the decrease in mCD93 expression and the increase in sCD93 secretion from U937 cells treated with Ni^{2+} are not fully understood at the present time, we are vigorously analyzing the detailed mechanism(s) concerning these immunological phenomena.

In conclusion, we observed a decrease in mCD93 expression and an increase in sCD93 secretion in U937 cells treated with Ni^{2+}. These findings strongly suggest that the modulation of CD93 molecules (both mCD93 and sCD93) may serve as a new biomarker for analyzing CHS with inflammation induced by Ni^{2+}. Further analyses are needed to elucidate the detailed intracellular signal transduction mechanism(s) of these findings at the molecular level.

References

1. Budinger, L., Hertl, M.: Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview. Allergy 55:108-115, 2000.
2. Rui, F., Bovenzi, M., Prodi, A., et al.: Nickel, cobalt and chromate sensitization and occupation. Contact Derm. 62:225-231, 2010.
3. Schafer, T., Bohler, E., Ruhdorfer, S., et al.: Epidemiology of contact allergy in adults. Allergy 56:1192-1196, 2001.
4. de Waard-van der Spek, F.B., Oranje, A.P.: Patch tests in children with suspected allergic contact dermatitis: A prospective study and review of the literature. Dermatology 218:119-125, 2009.
5. Brandão, M.H., Gontijo, B.: Contact sensitivity to metals (chromium, cobalt and nickel) in childhood. An. Bras. Dermatol. 87:269-276, 2012.
6. McGreal, E.P., Ikewaki, N., Akatsu, H., et al.: Human C1qRp is identical with CD93 and the mNI-11 antigen but does not bind C1q. J. Immunol. 168:5222-5232, 2002.
7. Greenlee, M.C., Sullivan, S.A., Bohlson, S.S.: CD93 and related family members: their role in innate immunity. Curr. Drug Targets 9:130-138, 2008.
8. Norsworthy, P.J., Fossati-Jimack, L., Cortes-Hernandez, J., et al.: Murine CD93 (C1qRp) contributes to the removal of apoptotic cells in vivo but is not required for C1q-mediated enhancement of phagocytosis. J. Immunol. 172:3406-3414, 2004.
9. Ikewaki, N., Kulski, J.K., Inoko, H.: Regulation of CD93 cell surface expression by protein kinase C isoenzymes. Microbiol. Immunol. 50: 93-103, 2006.
10. Bohlson, S.S., Silva R., Fonseca, M.I., et al.: CD93 is rapidly shed from the surface of human myeloid cells and the soluble form is detected in human plasma. J. Immunol. 175:1239-1247, 2005.
11. Greenlee-Wacker, M.C., Sullivan, S.A., Bohlson, S.S.: Detection and characterization of soluble CD93 released during inflammation. Inflamm. Res. 58:909-919, 2009.
12. Greenlee-Wacker, M.C., Galvan, M.D., Bohlson, S.S.: CD93: recent advances and implications in disease. Curr. Drug Targets 13:411-420, 2012.
13. Ikewaki, N., Yamao, H., Kulski, J.K., et al.: Flow cytometric identification of CD93 expression on naive T lymphocytes (CD4(+)CD45RA (+) cells) in human neonatal umbilical cord blood. J. Clin. Immunol. 30:723-733, 2010.
14. Park, M., Tenner, A.J.: Cell surface expression of C1qRP/CD93 is stabilized by *O*-glycosylation. J. Cell. Physiol. 196:512-522, 2003.
15. Ikewaki, N., Tamauchi, H., Inoko, H.: Decrease in CD93 (C1qRp) expression in a human monocyte-like cell line (U937) treated with various apoptosis-inducing chemical substances. Microbiol. Immunol. 51:1189-1200, 2007.
16. Ikewaki, N., Ohya, K.: Decrease of CD93 expression on the human monocyte-like cell line U937 treated with anti-Fas monoclonal antibody. J. Kyushu Univ. Health and Welfare 8: 123-131, 2007.
17. Yanaba, K., Asano, Y., Noda, S., et al.: Augmented production of soluble CD93 in patients with systemic sclerosis and clinical association with severity of skin sclerosis. Br. J. Dermatol. 167:542-547, 2012.
18. Schmidt, M., Raghavan, B., Müller, V., et al.: Crucial role for human toll-like receptor 4 in the development of contact allergy to nickel. Nat. Immunol. 11:814-819, 2010.
19. Raghavan, B., Martin, S.F., Esser, P.R., et al.: Metal allergens nickel and cobalt facilitate TLR4 homodimerization independently of MD2. EMBO Rep. 13:1109-1115, 2012.

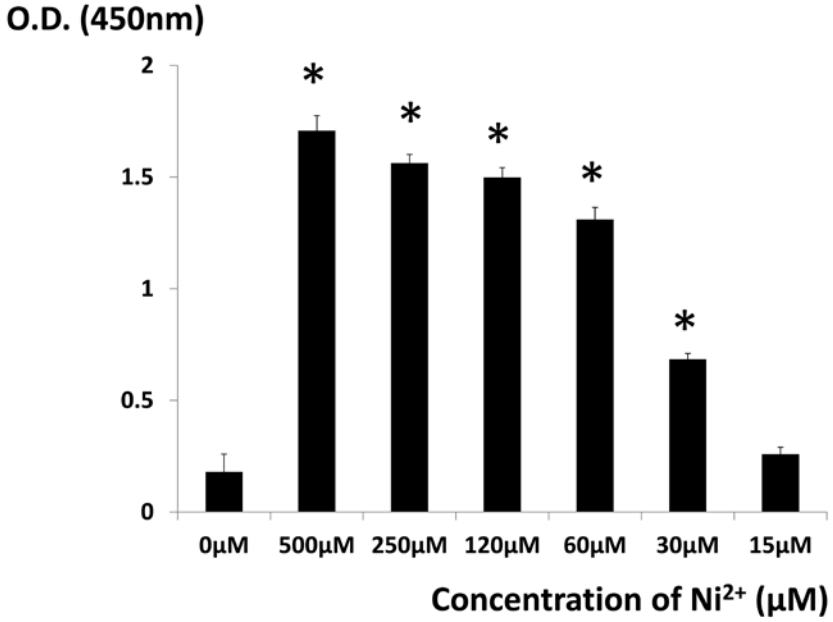

Figure 1. Induction of apoptosis in U937 cells treated with Ni^{2+}. Apoptotic monitoring of U937 cells treated with or without Ni^{2+} was performed using an apoptosis detection EIA kit (See Materials and Methods for details). Data were represented as the mean ± SD. The analysis was repeated in triplicate per sample. The experiment was repeated three times. *$P < 0.05$ (vs. 0 μM).

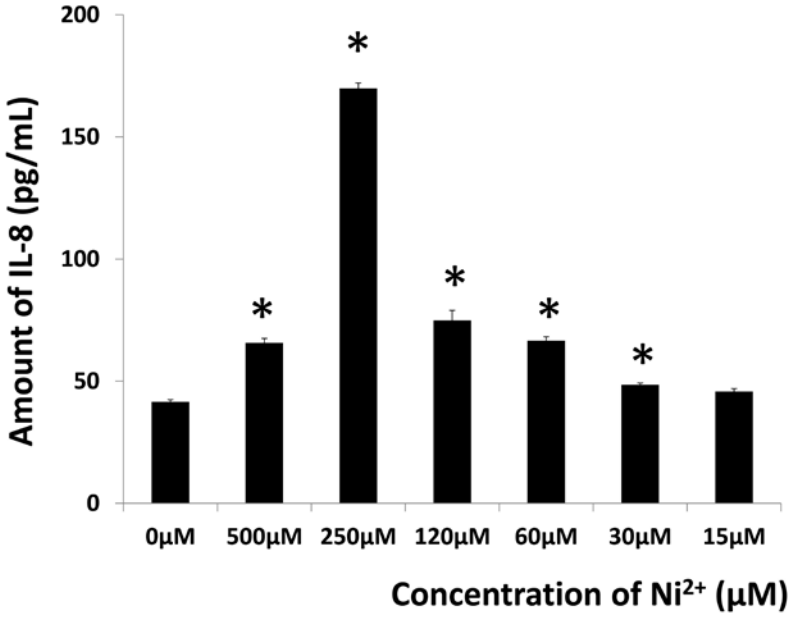

Figure 2. Enhancement of IL-8 secretion from U937 cells treated with Ni^{2+}. Detection of IL-8 in the supernatants from U937 cells treated with or without Ni^{2+} was performed using an IL-8 detection EIA kit (See Materials and Methods for details). Data were represented as the mean ± SD. The analysis was repeated in triplicate per sample. The experiment was repeated three times. *$P < 0.05$ (vs. 0 μM).

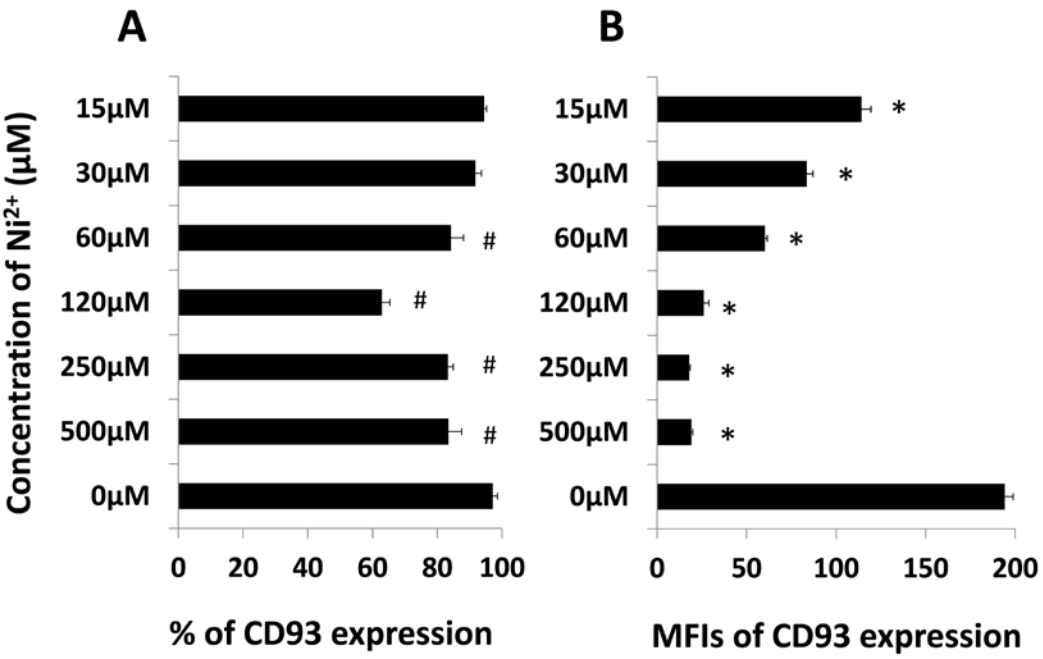

Figure 3. Decrease in mCD93 expression on U937 cells treated with Ni^{2+}. The percentages (panel A) and mean fluorescence intensities (MFIs) (panel B) of mCD93 expression on U937 cells treated with or without Ni^{2+} were analyzed using a single-color flow cytometric system (See Materials and Methods for details). Data were represented as the mean ± SD. The analysis was repeated in triplicate per sample. The experiment was repeated three times. #$P < 0.05$ (vs. 0 μM).*$P < 0.05$ (vs. 0 μM).

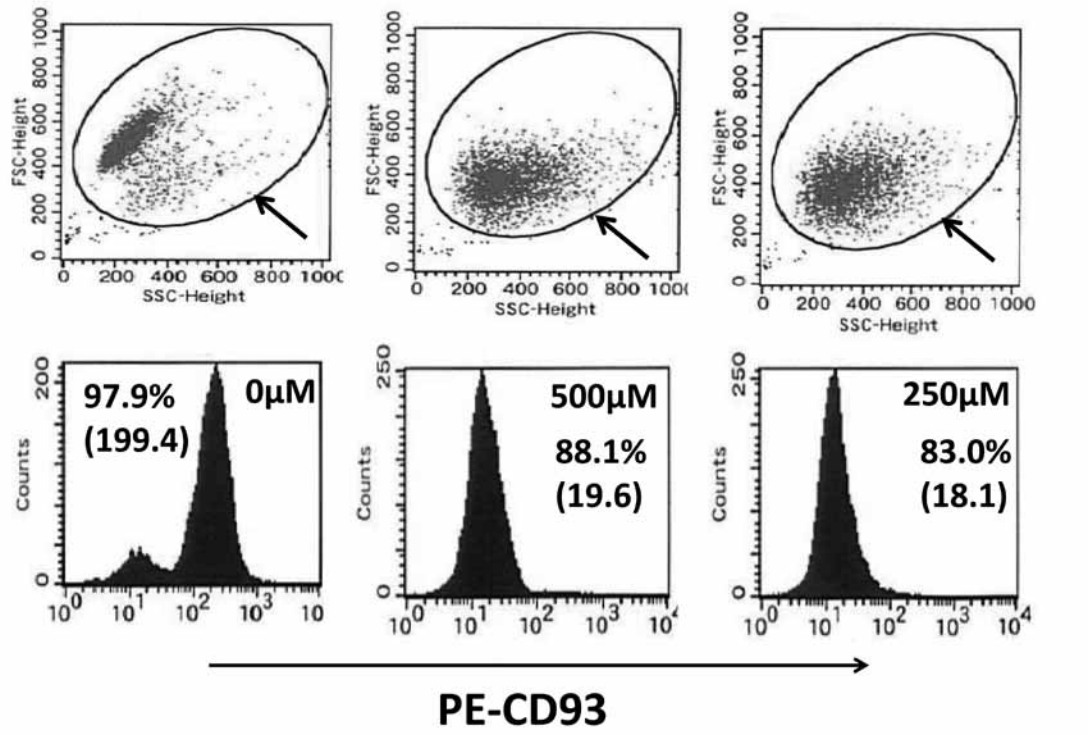

Figure 4. Typical flow cytometric profiles of mCD93 on U937 cells treated with Ni^{2+}. The percentages and mean fluorescence intensities (MFIs) of mCD93 expression on U937 cells treated with or without Ni^{2+} were analyzed using a single-color flow cytometric system (See Materials and Methods for details). The *arrow within the upper panel* indicates the analysis population. Percentages and MFIs are expressed in each panel. The values in parentheses are the MFIs.

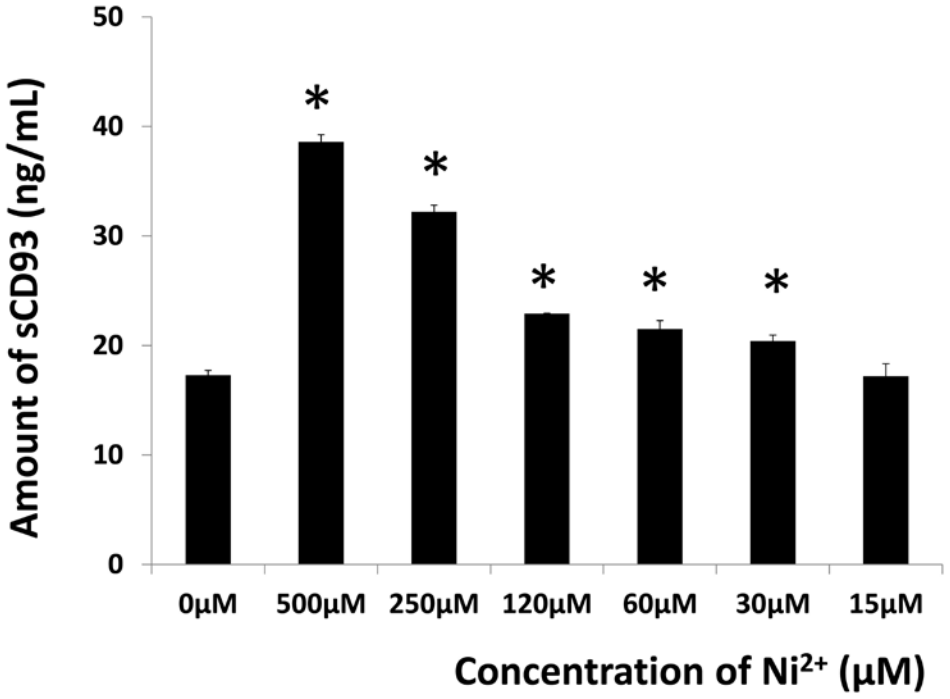

Figure 5. Increase in sCD93 secretion from U937 cells treated with Ni^{2+}. The detection of sCD93 in supernatants from U937 cells treated with or without Ni^{2+} was performed using an EIA system (See Materials and Methods for details). Data were represented as the mean ± SD. The analysis was repeated in triplicate per sample. The experiment was repeated three times. $^{*}P < 0.05$ (vs. 0 μM).

ニッケルで処理されたヒト単球系細胞株（U937）における CD93 分子の動態

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要　旨

ニッケルで処理されたヒト単球系細胞株（U937）における CD93 分子の動態を解析した。ニッケル処理は U937 細胞に対してインターロイキン -8(IL-8) の産生を増強しながらアポトーシスを誘導した。また、ニッケル処理により U937 細胞表面上の CD93 分子（mCD93）の発現は有意に減少し、可溶性 CD93 分子（sCD93）の産生は有意に増強した。以上の結果は、CD93 分子の動態が金属（ニッケル）アレルギーの発症メカニズムを解析するための新たなバイオマーカーに成り得ることを示唆している。

キーワード：CD93 分子、接触過敏、ニッケル、U937 細胞