

Effects of Nickel on Eosinophil Survival

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Key Words

Eosinophils · Metal · Nickel

Abstract

Background: Accessories, watches, coins and other items containing metal sometimes cause contact dermatitis and metal allergy. Among metals, nickel in alloys is ionized by sweat on the surface of the skin and exhibits particularly marked irritancy and allergenicity. Although eosinophils play important roles in allergy, the effects of nickel on eosinophils have not been elucidated. **Methods:** Eosinophils were prepared from the peritoneal cavity in rats immunized with *Ascaris suum* extract. Purified rat eosinophils were incubated in the presence of various kinds of metals including nickel. The viability of eosinophils was analyzed using a flow cytometer. **Results:** When rat eosinophils were incubated for 3 days in the presence of nickel chloride at 30–1,000 μM , the viability of eosinophils was decreased in a concentration-dependent manner. Nickel chloride at 300 μM significantly increased the percentage of annexin V⁺ PI[–] eosinophils. The population of annexin V⁺ PI[–] eosinophils was also increased by nickel sulfate, cobalt chloride and zinc sulfate. The binding of nickel ions to eosinophils was detected by flow cytometer. **Conclusions:** Nickel ions bind to eosinophils and decrease the viability of eosinophils through the induction of apoptosis. Nickel ions may exhibit activity which modifies the function of eosinophils in allergy.

Introduction

Accessories, watches, coins and other items containing metals sometimes cause contact dermatitis and metal allergy [1, 2]. Among metals, nickel in alloys is ionized by sweat on the surface of the skin and is characterized by irritancy and allergenicity. It has been reported that nickel challenge in the dermis of patients with contact allergy by the skin window technique revealed the infiltration of mononuclear cells, basophils and eosinophils [2, 3].

Eosinophils are suggested to play important roles in the pathogenesis of allergic inflammation such as bronchial asthma and atopic dermatitis [4, 5]. In patients suffering from these allergic diseases, the number of eosinophils in the bone marrow, peripheral blood and at inflammatory sites is increased because of their prolonged survival. Recently, it has been reported that asthmatic patients have a higher sensitivity to nickel than normal [6]. However, the effects of nickel on eosinophils have not been elucidated. In this study, therefore, we analyzed the effects of nickel on the viability of eosinophils.

Materials and Methods

Preparation and Culture of Rat Eosinophils

Preparation of rat peritoneal eosinophils was carried out as previously reported [7]. The purity of eosinophils was more than 95%, and the viability was more than 98%. Eosinophils were seed-

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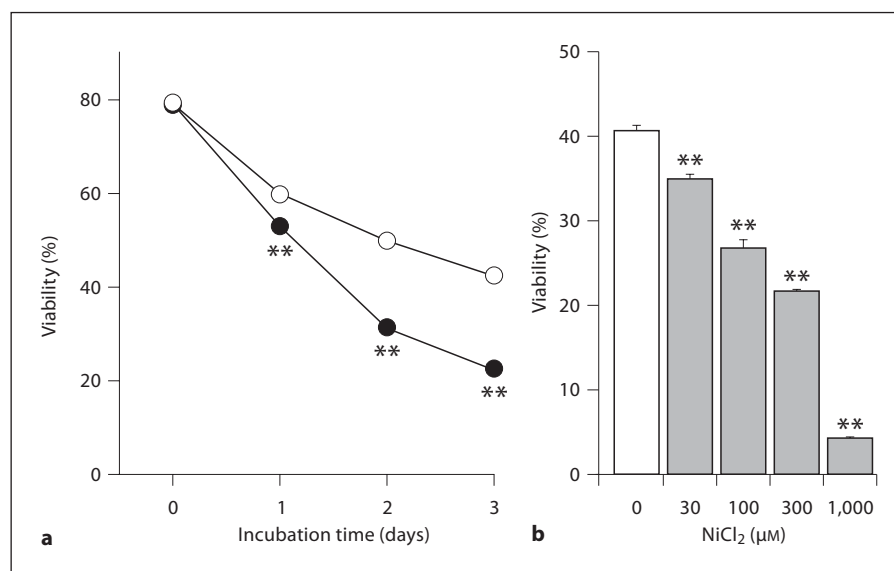
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Fig. 1. Effects of nickel chloride on the viability of eosinophils. Eosinophils were incubated at 37°C (**a**) for the periods indicated in medium in the absence (○) or presence of nickel chloride (300 μ M; ●) and (**b**) for 3 days in the presence of the indicated concentrations of nickel chloride. After the incubation, to determine the viability of eosinophils, the cells were stained with 7-AAD and analyzed by flow cytometer. Values are the means from 4 samples with SEM. SEM is within each symbol. Statistical significance: ** $p < 0.01$ vs. corresponding control.



ed in each well of 24-well plates at a density of 1×10^6 cells in 1 ml of 10% FBS-RPMI-1640 medium containing various kinds of metals. The metals compounds used in this experiment were nickel chloride, cobalt chloride, manganese chloride, nickel sulfate, zinc sulfate, and aluminum sulfate (Wako, Osaka, Japan). These metals were dissolved in water and added to the medium. The concentration of the vehicle in the medium was adjusted to 0.1% (v/v).

Flow Cytometry

After the incubation of eosinophils for various periods in the presence of metal compounds, the cells were stained with 7-aminoactinomycin D (Sigma, St. Louis, Mo., USA) for analysis of viability, or with FITC-labeled annexin V and propidium iodide (PI) (Sigma) for analysis of apoptosis for 5 min at room temperature. To detect binding of nickel ions to eosinophils, the cells were incubated in medium containing nickel chloride for 5 min at room temperature, washed with PBS three times, and further incubated with Newport Green DCF (Invitrogen, Carlsbad, Calif., USA) for 5 min at room temperature. After washing with PBS, the fluorescence of Newport Green binding on nickel ions and eosinophils was analyzed using a flow cytometer (FACScan; Beckton Dickinson, Franklin Lakes, N.J., USA).

Statistical Analysis

The statistical significance of the results was analyzed using Dunnett's test for multiple comparisons.

Results and Discussion

When rat eosinophils were incubated for 1, 2 and 3 days at 37°C, their viability was decreased time-dependently (fig. 1a). Nickel chloride further decreased the via-

bility of cells at each day (fig. 1a). Upon treatment with nickel chloride at 30–1,000 μ M, the viability of cells at 3 days was decreased by nickel chloride in a concentration-dependent manner (fig. 1b). To clarify whether the decrease in the viability of eosinophils by nickel chloride was due to the induction of apoptosis, we examined the annexin V⁺ PI⁻ population in eosinophils incubated for 1 day with medium containing 300 μ M of nickel chloride. As shown in figure 2, nickel chloride increased the percentage of annexin V⁺ PI⁻ eosinophils. These findings suggest that nickel chloride decreases the viability of eosinophils through the induction of apoptosis. Next, we analyzed whether other metal compounds at 300 μ M induce eosinophil apoptosis. Nickel sulfate induced apoptosis at almost the same level as nickel chloride, while that induced by cobalt chloride was moderate and that induced by zinc sulfate was weak compared with induction by nickel chloride and nickel sulfate (fig. 2). These results suggest that nickel ions, cobalt ions and zinc ions induce the apoptosis of eosinophils. It has been reported that nickel, cobalt and zinc are allergenic, and it is well known that nickel and cobalt cause allergy [1]. In contrast, manganese chloride inhibited apoptosis (fig. 2). Manganese is an essential element for all types of tissue, and plays important roles in the free radical defense system as manganese superoxide dismutase (MnSOD), which protects mitochondria from damage by superoxide radicals [8, 9]. Eosinophils have been reported to undergo apoptosis by reactive oxygen species (ROS) [10], and MnSOD is involved in the inhibition of eosinophil apoptosis [11].

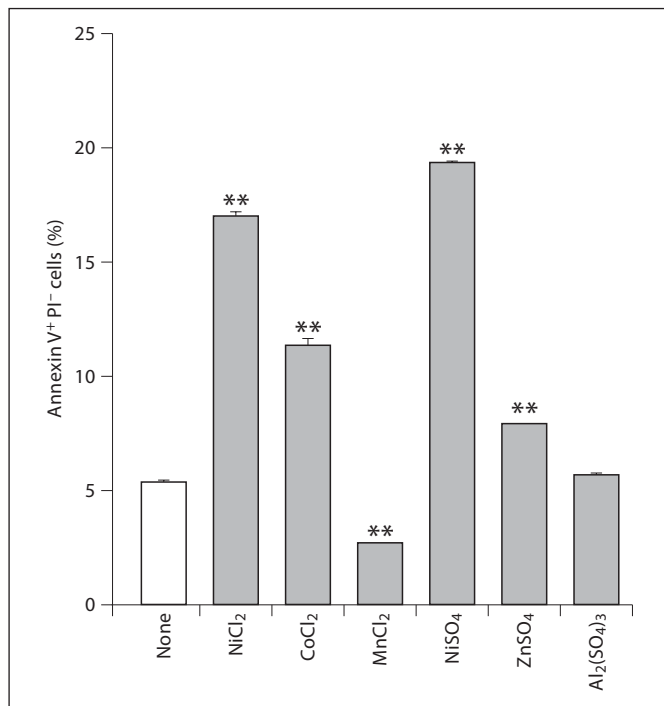


Fig. 2. Effects of various metal compounds on eosinophil apoptosis. Eosinophils were incubated at 37°C for 1 day in medium in the presence of 300 μ M of the indicated metal compounds. After the incubation, the cells were stained with FITC-labeled annexin V and PI and analyzed by flow cytometer. Values are the means from 4 samples with SEM. Statistical significance: ** $p < 0.01$ vs. corresponding control.

Therefore, it is possible that manganese inhibits eosinophil apoptosis via MnSOD. Taken together, our findings suggest that the metal specificity of the induction of eosinophil apoptosis is correlated with allergenicity.

We analyzed whether nickel ions bind to eosinophils, and detected an increase of fluorescence of Newport Green, which is a cell-impermeable fluorophore for nickel ions, on eosinophils by flow cytometry (fig. 3). Pretreatment of eosinophils with nickel chloride enhanced the fluorescence intensity, suggesting that nickel ions bind to the surface of eosinophils (fig. 3). It has been reported that nickel ions bind to numerous proteins including cell surface molecules, major histocompatibility complex (MHC) class I and MHC class II [12]. Therefore, it is possible that nickel ions bind not only to the molecules on the cell surface as shown in figure 3, but also to intracellular molecules in eosinophils.

It has been reported that nickel ions induce the production of ROS and prostaglandins, expression of Fas ligand, and various cytokines, such as IL-8, TNF- α and

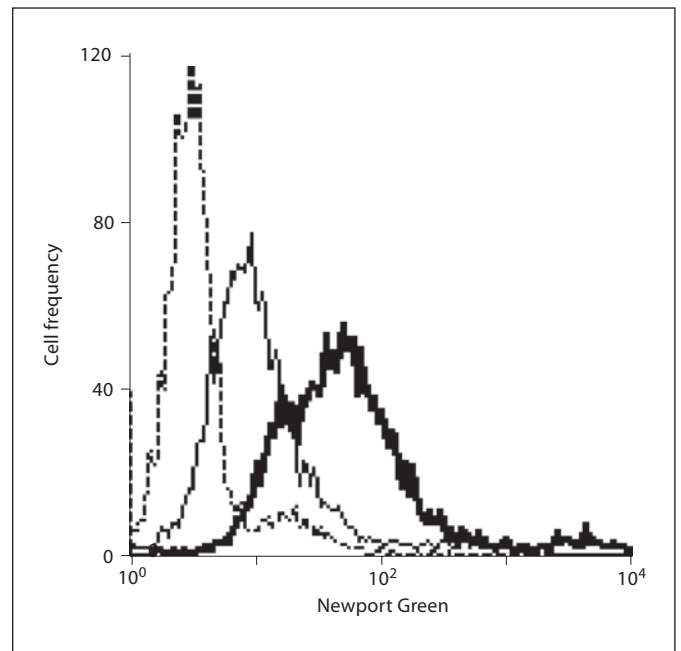


Fig. 3. Binding of nickel ions to eosinophils. Eosinophils were incubated in the presence (bold line) or absence (thin line) of 300 μ M of nickel chloride for 5 min and then with 1 μ M of Newport Green for 5 min at room temperature. After washing with PBS, the binding of nickel ions to eosinophils was analyzed by flow cytometer. The dashed line represents the fluorescence of cells incubated in the absence of nickel chloride and Newport Green. The experiment shown is representative of 4 independent experiments.

TGF- β , via activation of signal transduction molecules including mitogen-activated protein kinases [13–19]. In these molecules, ROS, Fas ligand and TGF- β are reported to induce apoptosis of eosinophils. Therefore, it is suggested that eosinophils undergo apoptosis by nickel ions through the production of these molecules. In contrast, infiltration of eosinophils toward inflammatory sites is induced by nickel in patients with contact allergy [3] and bronchial asthma [6], and it is thought that eosinophils play important roles in the pathogenesis of allergies. However, this study indicated that eosinophil viability is decreased by nickel ions (fig. 1b). In patients, nickel ionized by sweat on the surface of the skin induces the expression of various cytokines at the inflammatory sites [2, 13–19]. IL-8, which is a chemotactic factor for eosinophils, produced by nickel ions from epithelial cells [16] might be involved in the infiltration of eosinophils into the sites. Eosinophils might also produce inflammatory mediators such as cytokines and lipids involved in the pathogenesis of allergies on exposure to nickel ions. Thus,

it is possible that simultaneous signaling for the expression of various inflammatory mediators from eosinophils and that for the induction of apoptosis in eosinophils might be involved in the inflammation and allergy induced by nickel in vivo. Therefore, eosinophils might play important roles for the nickel-induced allergy as effector cells.

In the study of metal allergy, T cells have been focused on because metal allergy is recognized as being a type IV allergy. However, nickel ions were found to act on eosinophils in the present study. Because it has been unknown whether nickel ions induce the expression of mediators from eosinophils, further investigation is necessary for the implication of eosinophils in metal allergy.

In conclusion, we suggest that nickel may exhibit a modifying activity on the function of eosinophils in allergy.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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