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Enhancement of nickel elution by lipopolysaccharide-induced inflammation

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ABSTRACT

Background: Implantations of metallic biomedical devices into bodies are increasing. The elution of Ni ions from these devices can lead to metal allergies. However, the molecular mechanisms of the elution have not been fully examined. Furthermore, it is not clear whether infection and inflammation affect the corrosion of metals.
Objective: We examined whether the elution of Ni from metal wires and plates was enhanced by inflammation *in vivo* and *in vitro*.
Methods: A Ni or SUS316L wire was implanted subcutaneously in the dorsum of mice. Lipopolysaccharide (LPS) was injected at the site immediately following the implantation. After 8, 24, and 72 h, the tissue around the wire was excised. RAW 264 cells were seeded on a Ni plate and incubated for 24 h in medium containing LPS. The amount of Ni in the tissue or conditioned medium was determined fluorometrically.
Results: The release of Ni ions from the wire was significantly increased from 8 to 72 h, and further increased by LPS. LPS also enhanced the release of Ni ions by the cells, but only when they were attached to the Ni plate. Chloroquine, bafilomycin A_1 and amiloride markedly inhibited the effects of LPS.
Conclusion: The activation of inflammatory cells on metals enhanced the elution of Ni probably via the release of protons at the interface of the cells and material.

1. Introduction

Implantations of biomedical devices to treat diseases and organ insufficiencies are increasing as populations age [1]. Most devices for the replacement of hard tissues, including artificial hip joints, bone plates and dental implants, comprise metallic biomaterials because of their reliable mechanical performance [2,3]. Corrosion-resistant and ductile, nickel (Ni) is contained in various alloys, including stainless steels, Ni–Cr and Ni–Ti. However, Ni is also the most common contact allergen among metals [4]. Allergies to Ni, classified as Type IV allergies [5], are initiated by the release of Ni ions from alloys. Ni bound to soluble proteins [4] or to proteins on antigen-presenting cells [6] is recognized as an antigen. The antigen-presenting cells then activate T cells and induce an increase in the number of Ni-specific, IFN-γ-producing $CD4^+$ and $CD8^+$ effector T cells [7]. It is difficult to prevent Ni allergies by inhibiting these immune responses. A more practical approach might be to block the elution of Ni ions from biomaterials. However, the molecular mechanisms of this elution have not been fully examined. Furthermore, although the inflamed sites become acidic, it is not clear whether infection and inflammation affect the corrosion of metals.

In general, the release of Ni ions from alloys is tested in solutions [8,9]. However, as biodevices are implanted for long periods of time, one should test the release of metal ions from alloys in tissues to assess the risk of inducing metal allergies. There is little evidence that the release of metal ions from alloys *in vivo* is similar to that *in vitro*.

To reveal the mechanisms of Ni-induced inflammation and allergy, several animal models have been developed. The injection of Ni ions into sensitized animals was found to cause allergic inflammation including ear swelling [8–10], footpad edema [11] and the proliferation of lymph node cells [12]. Tolerance to nickel sensitization was also examined in these animals [9]. Recently, Sato et al. found that the co-administration of lipopolysaccharide (LPS), a stimulator of innate immune responses [13], effectively enhanced sensitization to Ni [10]. Sensitization using Ni ions plus LPS will promote research into how metal allergies are evoked. In contrast to models using Ni ions, we induced inflammation in mice using a Ni

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wire [14]. In this model, Ni wire but not Fe, Al and Co wires induced necrosis of the surrounding tissues. The implantation of the Ni wire elicited the increase in plasma exudation 8 h after the implantation and prostaglandin and histamine mediated the responses, indicating that Ni ions were released within a few hours [14].

LPS mimics the inflammation induced by an infection, which can occur when a biomedical device is implanted [15,16]. Therefore, it is possible that the injection of LPS enhances the elution of Ni ions from metals. In this study, using Ni wire-induced inflammation model and a novel *in vitro* system, we examined the mechanisms responsible for the elution of Ni.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice, specific pathogen free and weighing 22–25 g, were purchased from SLC (Shizuoka, Japan). The mice were treated in accordance with procedures approved by the Animal Ethics Committee of Tohoku University, Japan.

2.2. Materials

A Ni wire (>99%, ϕ 0.8 mm), a SUS316L wire (ϕ 1 mm), and a Ni plate (>99%, thickness: 0.05 mm) were purchased from Nilako Co., Tokyo, Japan. Lipopolysaccharide (LPS) was purchased from Sigma–Aldrich (St. Louis, MO) and amiloride, chloroquine, and bafilomycin A_1 were from Wako Pure Chemical Ind. (Osaka, Japan).

2.3. Implantation of the Ni wire into mice

A nickel wire and a SUS316L wire were cut into 5 mm lengths and sterilized by soaking in ethanol. Mice were anesthetized and a length of wire was implanted subcutaneously in the dorsum using a 13G implant needle (Natsume Co., Tokyo, Japan). A hundred microliters of LPS solution (1 μg/ml) or saline was injected into the site immediately after the implantation. The mice were sacrificed at the indicated time and then the skin tissue (diameter: 14 mm) on the wire was excised. The skin samples were minced with scissors in 500 μl of Milli Q water and left at 4 °C overnight. The extract was centrifuged at 15,000 rpm for 20 min, and the supernatant was used to determine Ni concentrations as described below.

2.4. Determination of Ni concentrations by fluorometry and ICP-AES

Samples were diluted with Milli Q water, and Newport Green DCF [17] was added (150 μl per tube: 0.75 μM) to 850 μl of diluted sample. Fluorescence intensity was determined with excitation and emission wavelengths of 505 and 535 nm, respectively, with the use of a Fluorometer F-2000 (Hitachi High-Technologies Corporation, Japan). The Ni concentrations of some samples were determined by inductively coupled plasma atomic emission (ICP-AES) with an ICPS-8000 (Shimadzu System Development Corporation, Japan).

2.5. Macroscopic and histochemical analyses

Mice were sacrificed 0, 8, 24, or 72 h after the implantation. Tissue (14 mm in diameter) including epidermis, dermis and subcutaneous tissue on the wire was then excised and weighed. The wires obtained were washed with phosphate-buffered saline and then with an ice-cold lysis buffer (20 mM HEPES, pH 7.4, 1% (v/v) Triton-X 100, 10% (v/v) glycerol) to remove attached cells. The surface of wires was observed by scanning electric microscope (S-3200N, Hitachi, Japan).

2.6. Incubation of the Ni wire in vitro

A Ni wire was incubated in 50 μl of Milli Q water, saline, or mouse serum at 37 °C for 8 h. Then, the amount of Ni eluted was determined as described above.

2.7. Cell culture and stimulation

RAW 264 cells, mouse macrophage cell lines, were cultured at 37 °C under 5% CO_2–95% air in phenol red-free Eagle's minimal essential medium containing 10% (v/v) fetal bovine serum, penicillin G potassium (15 μg/ml), and streptomycin sulfate (50 μg/ml). The cells were suspended at the indicated numbers in the same medium, and 200 μl of the cell suspension was added to a Ni plate (25 mm^2) in each well of a 96-well cluster dish. Two hours later, LPS was added at the indicated concentrations and the cells were further incubated at 37 °C for 24 h or the period indicated. To prevent them attaching to plates, the cells were seeded first, and 2 h later, the Ni plate was placed in a sloping position into each well.

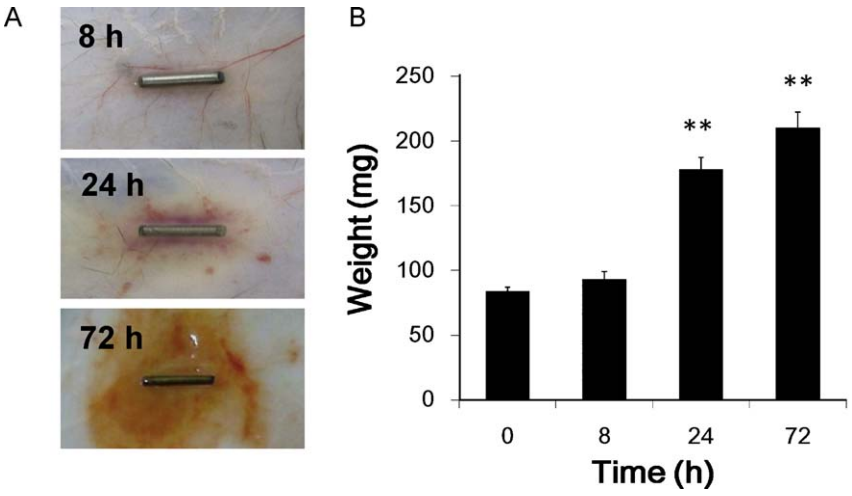

Fig. 1. Ni wire-induced inflammation. (A) A Ni wire was implanted subcutaneously in the dorsum of each mouse. The mice were sacrificed 8, 24 and 72 h after the implantation, and the skin around the wire was photographed. (B) The skin tissue (diameter: 14 mm) on the wire was excised and the weight of tissue was measured. The values are means for four mice with the S.E.M. shown by vertical bars. Statistical significance: $^{**}P < 0.01$ vs. the 0 h group.