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Nickel acts as an adjuvant during cobalt sensitization

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Background

Nickel allergy is common and nickel allergic individuals are often allergic to cobalt as well. Concomitant sensitization to nickel and cobalt appears to increase the severity of metal induced allergic hand dermatitis when compared to sensitization to either cobalt or nickel alone (1). This observation indicates that exposure and primary sensitization to nickel and/or cobalt may play an important role for the subsequent clinical immune response to these metals. Despite the high prevalence of concomitant nickel and cobalt allergy (1), there appears to be minimal cross-reactivity of human nickel-specific T cells when exposed to cobalt in vitro (2,3). Likewise, animal studies have supported the notion that nickel- and cobalt-specific T cells do not cross-react (4). Thus, other factors must play a role in nickel-cobalt co-sensitization for example co-exposure.

It has been demonstrated that addition of an irritant such as sodium lauryl sulfate during challenge with nickel leads to a stronger reaction against nickel in patients with nickel allergy (5). Notably, an increased inflammatory response mediated by an irrelevant allergen or irritant can lower the activation threshold of the specific allergen during the challenge reaction (6). In line with this, we recently showed that sensitization with a mixture of fragrance allergens leads to a stronger challenge response to specific fragrance allergens in the mixture when compared to sensitization with the specific allergen alone (7). Furthermore, animals sensitized with nickel and cobalt appeared to react more readily in patch testing to either allergen as compared to mono-sensitized animals (8). Collectively, these data suggest that there might exist a 'cocktail-effect', where the presence of additional allergens/irritants during the sensitization phase to a specific allergen can induce a stronger activation of the specific im-

immune response and thereby increase the allergic response during subsequent exposures to the specific allergen. Here, we further explored whether a ‘cocktail-effect’ exist between nickel and cobalt during sensitization to these allergens.

Question addressed

Does sensitization with a mixture of nickel and cobalt enhance the specific immune response following subsequent challenge with either nickel or cobalt alone?

Experimental design

Mice were sensitized with 10% NiCl₂, 10% NiCl₂ plus 1% CoCl₂, 10% CoCl₂, 10% CoCl₂ plus 1% NiCl₂ in white petrolatum or vehicle (pure white petrolatum) as previously described (Figure 1A) (9). Mice studies were performed in accordance with Danish national animal protection guidelines Three weeks after sensitization, the mice were challenged by painting with 10% NiCl₂, 10% CoCl₂ or vehicle (pure white petrolatum) twice with a 24 h interval. To detect T and B cell proliferation, mice were given the thymidine analog 5-bromo-2-deoxyuridine (BrdU) (0.8 mg/mL) in the drinking water on the first day of challenge. Mice were euthanized 48 h after the first challenge. Ear thickness was measured in a blinded manner using an engineer’s micrometer and the cellular immune response within the draining lymph nodes was analyzed by flow cytometry.

Results

Greater ear-swelling was induced by challenge with nickel compared to challenge with cobalt in mice that had been exposed to petrolatum during sensitization, indication that nickel is a stronger irritant than cobalt (Fig. 1B, group b and e). In mice that were both sensitized and challenged with nickel (Fig.

1B, group c) an increased ear-swelling was seen compared to mice that were sensitized with vehicle and then challenged with nickel (Fig. 1B, group b). Likewise, for mice that were both sensitized and challenged with cobalt (Fig. 1B, group f), increased ear-swelling was seen compared to mice that were sensitized with vehicle and then challenged with cobalt (Fig. 1B, group e). A 'cocktail-effect' appeared to exist between nickel and cobalt when nickel was included during cobalt sensitization (Fig. 1B, group g) whereas the addition of cobalt to nickel during sensitization did not change the ear-swelling induced by challenge with nickel (Fig. 1B, group d). Moreover, the addition of nickel during cobalt sensitization led to a significant increase in the number of proliferating B cells and CD4⁺ T cells following challenge with cobalt when compared to mice sensitized with cobalt alone (Fig. 2A and B). Interestingly, addition of cobalt during nickel sensitization only had a minor effect on the number of proliferating B cells and CD4⁺ T cells induced by nickel challenge (Fig. 2A and B). In contrast, addition of cobalt during the sensitization with nickel caused a significant increase in the number of proliferating CD8⁺ T cells following nickel challenge compared to mice sensitized with nickel alone (Fig. 2C). In parallel, addition of nickel during cobalt sensitization also led to a significant increase in the number of proliferating CD8⁺ T cells following challenge with cobalt compared to mice sensitized with cobalt alone (Fig. 2C).

Conclusions

We show that a mixture of nickel and cobalt during the sensitization phase enhances the specific immune response to both metals following challenge with either nickel or cobalt alone. Both nickel and cobalt sensitization led to an increased ear-swelling with the highest values induced by nickel suggesting that nickel is a stronger irritant than cobalt. Following cobalt challenge, an increased ear-swelling

as well as increased proliferation of B cells and T cells in the draining lymph node was seen in mice sensitized with cobalt in the presence of nickel compared to mice sensitized with cobalt alone. The presence of cobalt during sensitization to nickel resulted only in an increased proliferation of CD8⁺ T cells compared to mice sensitized only with nickel, whereas neither the local response nor the B cell and CD4⁺ T cell response were affected by the presence of cobalt during sensitization to nickel. In conclusion, our study demonstrates that a cocktail effect exists between nickel and cobalt, that nickel acts as a strong adjuvant during cobalt sensitization, and that co-exposure to nickel and cobalt should be avoided to limit metal allergy and dermatitis.

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Author contribution

CMB designed the study and performed the research, analyzed the data and wrote the paper, MMN performed the research and analyzed the data, MTV performed the research, JDJ designed the study and critically reviewed the manuscript, CG designed the study and critically reviewed the manuscript, JPT designed the study and wrote the paper.

Figure Legends

Figure 1. Nickel acts as an irritant and the presence of nickel during cobalt sensitization increases the subsequent challenge response to cobalt

(A) Schematic model of the experimental setup. (B) Ear thickness in mice that were sensitized and challenged as indicated. Data represent two independent experiments with 8 mice per group in each experiment. Statistical significance was evaluated by Student's unpaired two-tailed t-test (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Data are shown as mean + SD.

Figure 2. The presence of nickel during cobalt sensitization increases the number and proliferation of B cells and T cells in the draining lymph nodes during the subsequent challenge response to co

(A) Number of proliferating B cells (CD19⁺BrdU⁺ cells), (B) CD4⁺ T cells (CD4⁺BrdU⁺ cells) and (C) CD8⁺ T cells (CD8⁺BrdU⁺ cells) in the draining lymph nodes of mice that were sensitized and challenged as indicated. Data represent two independent experiments with 8 mice in each group. Statistical significance was evaluated by Student's unpaired two-tailed t-test (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$). Data are shown as mean + SD.

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