

The flare-up reactions after systemic provocation in contact allergy to nickel and gold

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In a controlled double-blind experimental study, patients with contact allergy to nickel or gold were exposed systemically to their contact allergen. Nickel sulfate was given orally, gold sodium thiomalate intramuscularly, and placebo by both routes. Clinical reactions including body temperature were followed and 7 plasma cytokines and acute phase reactants were assayed before and 24 h after provocation. Clinical flare-up, with reactivation of healing patch tests and/or toxicoderma-like reactions, was observed in patients receiving their specific allergen. A transient fever was noted in patients allergic to gold when exposed to gold, not in others. There was a significant increase in plasma tumour necrosis factor- α , soluble tumour necrosis factor receptor 1, interleukin-1 receptor antagonist, and neutrophil gelatinase associated lipocalin in patients allergic to gold when exposed to gold allergen. Furthermore, in patients allergic to nickel exposed to nickel allergen there was a highly significant increase in plasma soluble tumour necrosis factor receptor 1. Interferon-gamma, interleukin-4, and C-reactive protein did not increase in any of the patient groups. Clinical reactions during flare-up in contact allergy are accompanied by release in the blood of several cytokines. This release is not a consequence of a toxic effect or bound to a particular allergen. Instead, the cytokine release should be considered a general phenomenon characteristic of the flare-up in contact allergy.

Key words: nickel allergy; gold allergy; systemic provocation; flare-up; fever; cytokines; TNF- α ; sTNF-R1; IL-1 ra; NGAL © Munksgaard, 1999.

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The clinical picture of allergic contact dermatitis usually consists of a primary eruption at the site of allergen contact and occasionally secondary eruptions on other parts of the body. The location of secondary eruptions may be explained by different pathogenetic mechanisms. One possible explanation is by way of systemic exposure to the antigen, be that ingested, inhaled, or injected. Most frequent among experimental secondary eruptions are a flare-up of a healing or quiescent contact dermatitis as well as a toxicoderma-like rash. This has been demonstrated in contact allergy to, e.g., nickel (1) and gold (2); for the latter, fever reactions were also common. We have recently shown that the clinical reactions after parenteral provocation with a gold salt in patients with gold allergy are accompanied by immunological tissue reactions (3), as well as by release in blood of cytokines and acute phase reactants (4).

In the controlled study with gold provocation, the active agent, gold sodium thiomalate (GSTM), was clearly responsible for the clinical reactions.

Since, however, only patients with a contact allergy to gold were studied, a pharmacotoxic effect of the gold salt could not be ruled out. It might also be questioned if the clinical, morphological, and biochemical effects of systemic provocation were specific for gold salt as an antigen or if the findings were principal and characteristic for other antigens as well. Associated with this question is of course that of antigen specificity.

In order to answer the questions raised above, we have conducted a controlled, experimental study on patients allergic to gold or nickel. They were provoked either with their particular antigen, or the one to which they were not allergic, or with placebo. Clinical, morphological, and biochemical reactions were registered.

Materials and Methods

Patients with eczematous dermatitis, patch tested during the last 2 years, were invited to participate in a controlled study on the local and general ef-

fects of systemic exposure to a contact allergen. Permission was obtained from the Ethics Committee of Lund University Medical Faculty, and the patients gave informed consent. The aim was to find 20 patients allergic to gold but not to nickel, and 30 patients allergic to nickel but not to gold. In our standard patch test series, the marker for gold contact allergy is gold sodium thiosulfate (GSTS) 0.5 and 2.0% pet. and, for nickel allergy, nickel sulfate 5.0% pet. Patients with a contact allergy to both gold and nickel were avoided.

Patients consenting to the study were first patch tested with 2 dilution series (aq.), 1 with GSTS, starting at 5.0%, and 1 with nickel sulfate, starting at 16.0%, both in 9 reducing concentrations with a dilution factor of $\sqrt{10}$. Patches were applied on the back with Finn Chambers® on Scanpor® and removed after 48 h. Test reactions were read on day (D) 7. At this stage, some patients with a previously demonstrated nickel allergy had to be discarded because, in the meantime, they had developed a gold allergy as well. The reverse had not happened. 1 patient was excluded because a previously diagnosed nickel allergy could not be verified. In all patients with a previously positive patch test to gold, this could once again be demonstrated. The final patient material consisted of 20 patients with gold allergy, 13 females and 7 males, 29–72 years of age, and 28 patients with nickel allergy, all females, 19–75 years of age.

The experimental design was a double-blind, double-dummy, parallel group study. The intramuscular preparation was GSTM (Myocrisin® 20 mg/ml Rhône-Poulenc Rorer, Helsingborg, Sweden), alternatively riboflavin 0.1 mg/ml in 0.9% sodium chloride as placebo, given in a dose of 0.5 ml. The oral preparation was an opaque gelatine capsule containing either 11.2 mg of nickel sulfate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$), corresponding to 2.5 mg of nickel, in lactose, or lactose only as placebo. Randomization, formulation, and packaging of the study drugs was performed at the Hospital Pharmacy and each preparation was labelled with a patient number.

Immediately after reading of the patch tests at 9

a.m., the body temperature was measured intra-orally and a venous blood sample withdrawn, centrifuged and freeze-stored. The patients were divided according to Table 1 into 5 experimental groups (the diagnosis of gold or nickel allergy being known to the investigators but not the contents of the preparations for provocation). Thus, all patients obtained an i.m. injection (gold or placebo) and an oral capsule (nickel or placebo).

4 h after the systemic provocation 1–2 3-mm punch biopsies were taken from a positive patch test reaction. A 2nd biopsy was taken 24 h after the provocation from an adjacent test reaction and, at the same time, another venous blood sample. All skin biopsies were immediately snap frozen in liquid nitrogen. The body temperature was measured at home by the patient at 12 h and at the office after 24 h using one and the same, individual thermometer.

Laboratory studies

Enzyme-linked immunosorbent assay of neutrophil gelatinase associated lipocalin (NGAL) and soluble tumour necrosis factor receptor type 1 (sTNF-R1) were performed as earlier described (5, 6). Tumour necrosis factor- α (TNF- α), interleukin-4 (IL-4), interferon- γ (IFN- γ), and interleukin-1 receptor antagonist (IL-1 ra) were measured with ELISA kits (Quantikine™ from R&D Systems, Minneapolis, USA). C-reactive protein (CRP) was measured by electroimmunoassay (7).

Statistics

Analysis of intergroup differences of body temperature increase was carried out by the Kruskal-Wallis test; for results of the cytokine assays the Wilcoxon signed-rank test was used.

Results

Clinical reactions to the systemic provocations were few in the present study (Table 1). 3 out of 9

Table 1. The contents of systemic exposure in 5 patient groups allergic to nickel or gold and the clinical reactions

Group	Allergy	n	I.m.inj.	Oral capsule	Flare-up	Toxicoderma
1	nickel	9	placebo	nickel	3	–
2	nickel	9	gold	placebo	–	–
3	gold	10	placebo	nickel	–	–
4	gold	10	gold	placebo	6	1
5	nickel	10	placebo	placebo	–	1

Flare-up=positive patch test to more dilution steps than before provocation and/or reactivation of a previous dermatitis. Toxicoderma in group 4 designates a case of oedema of the hands and fingers, in group 5 of urticaria.

patients with a contact allergy to nickel had a flare-up of positive patch tests and/or of a previous eczematous infiltrate when provoked orally with nickel sulfate. Likewise, 6 out of 10 patients with gold allergy had an eczematous flare-up when injected with GSTM. There was no flare-up of contact dermatitis in the other 3 experimental groups. There were 2 cases of "toxicoderma": 1 gold allergy patient had, in addition to an eczematous flare-up, an oedematous reaction of the hands and fingers, starting 8–10 h after the GSTM injection; 1 patient with nickel allergy, after having placebo, had a bout of urticaria.

In 2 patients with gold allergy and an eczematous flare-up after gold provocation (Table 1, group 4), additional reactions were noted: 1 complained of generalized ache but particularly in the left cheek; she had experienced the same type of ache some years previously when she had had a golden dental bridge fitted. Another patient had abdominal pain and diarrhea 6 h after the GSTM injection.

Only slight changes in body temperature were noted from time 0 to 12 h (Fig. 1), mainly in group 4 (gold allergy patients provoked with gold). The mean rise in this group was statistically different from the other 4 groups ($p < 0.01$). The 3 patients with the highest fever in group 4 all had an eczematous flare-up, including the 1 with cheek ache and the 1 with hand oedema. The patient in group 5 with a fever reaction (after placebo) just felt ill in general and had no cutaneous flare-up.

Biochemical reactions

The biochemical reactions are summarized in Table 2. A significant increase was seen for TNF- α , sTNF-R1, IL-1 ra and NGAL in patients allergic to gold when exposed to the gold allergen

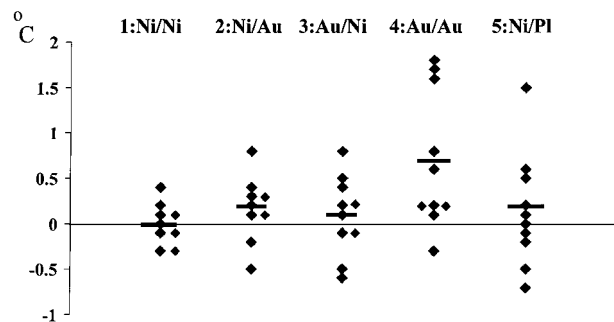


Fig. 1. Individual and mean change in body temperature from time 0 to 12 h after systemic provocation in 5 patient groups: Ni/Ni=allergic to nickel, provoked with nickel; Ni/Au=allergic to nickel/provoked with gold; Au/Ni=allergic to gold, provoked with nickel; Au/Au=allergic to gold/ provoked with gold; Ni/Pl=allergic to nickel/ provoked with placebo.

Table 2. The plasma level (mean $\pm$ SD) of 4 cytokines in 5 experimental groups before and 24 h after systemic provocation

	0 h	24 h	p-value
Group 1 (n=9): allergy to nickel, provoked with nickel			
TNF- α pg/ml	5.3 $\pm$ 2.3	5.5 $\pm$ 2.4	>0.05
sTNF R1 ng/ml	1.1 $\pm$ 0.7	1.6 $\pm$ 1.4	<0.005**
IL-1 ra pg/ml	433 $\pm$ 266	497 $\pm$ 216	>0.05
NGAL μ g/l	96 $\pm$ 22	95 $\pm$ 24	>0.05
Group 2 (n=9): allergy to nickel, provoked with gold			
TNF- α pg/ml	3.2 $\pm$ 1.0	3.2 $\pm$ 0.9	>0.05
sTNF R1 ng/ml	1.0 $\pm$ 0.1	1.1 $\pm$ 0.2	>0.05
IL-1 ra pg/ml	689 $\pm$ 589	813 $\pm$ 676	>0.05
NGAL μ g/l	96 $\pm$ 46	92 $\pm$ 26	>0.05
Group 3 (n=10): allergy to gold, provoked with nickel			
TNF- α pg/ml	4.1 $\pm$ 1.4	4.5 $\pm$ 2.0	>0.05
sTNF R1 ng/ml	1.2 $\pm$ 0.3	1.4 $\pm$ 0.3	>0.05
IL-1 ra pg/ml	914 $\pm$ 1329	1213 $\pm$ 1383	>0.05
NGAL μ g/l	90 $\pm$ 30	99 $\pm$ 29	>0.05
Group 4 (n=10): allergy to gold, provoked with gold			
TNF- α pg/ml	4.5 $\pm$ 1.2	6.0 $\pm$ 2.2	0.0326*
sTNF R1 ng/ml	1.1 $\pm$ 0.1	1.4 $\pm$ 0.3	0.0249*
IL-1 ra pg/ml	405 $\pm$ 184	733 $\pm$ 414	0.0166*
NGAL μ g/l	101 $\pm$ 28	123 $\pm$ 46	0.0218*
Group 5 (n=10): allergy to nickel, provoked with placebo			
TNF- α pg/ml	3.7 $\pm$ 1.8	3.8 $\pm$ 1.3	>0.05
sTNF R1 ng/ml	1.0 $\pm$ 0.3	1.1 $\pm$ 0.3	>0.05
IL-1 ra pg/ml	492 $\pm$ 255	494 $\pm$ 236	>0.05
NGAL μ g/l	98 $\pm$ 25	105 $\pm$ 32	>0.05

(group 4) but not in other groups. sTNF R1 showed a highly significant increase in patients allergic to nickel when exposed to the nickel allergen (group 1). IFN- γ , IL-4 and CRP did not increase in any of the patient groups.

The immunohistochemical findings in flaring patch test reactions will be the subject of a separate report.

Discussion

The present study confirmed our previous findings (2) when a gold salt was given systemically to patients with a contact allergy to gold: in many patients a flare-up of a previous or almost healed contact dermatitis was observed and, in addition, some other clinical reactions (Table 1, group 4). Furthermore, the specific provocation induced a small but highly significant increase in body temperature in this experimental group only (Fig. 1).

In our assay of 7 plasma cytokines and acute phase reactants, similar results to our recent study (4) were obtained: there was a significant increase in plasma TNF- α , sTNF R1, IL-1ra, and NGAL in gold allergy patients provoked with GSTM (Fig. 2, group 4). Epidermal keratinocytes might very well be the source of the TNF- α release, since the cytokine is rapidly produced from these cells when eliciting an allergic contact dermatitis (8, 9). Also,

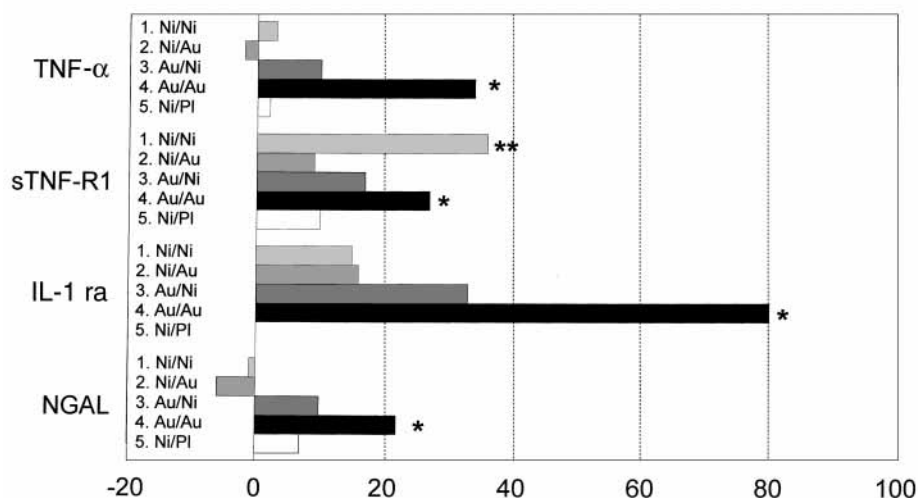


Fig. 2. Percentual change of 4 plasma cytokines from time 0 to 24 h after systemic provocation in 5 patient groups (see Fig. 1 for abbreviations). TNF α =tumour necrosis factor α ; sTNF-R1=soluble tumour necrosis factor receptor 1; IL-1 ra=interleukin 1 receptor antagonist; NGAL=neutrophil gelatinase associated lipocalin. Statistically significant changes are indicated (* $p < 0.05$, ** $p < 0.01$).

IL-1 seems to play an important role in contact allergy reactions (10), while the release of NGAL is more difficult to explain within the scenario of a regular contact dermatitis. The same result was, however, obtained in our previous study (4) and it should be underlined that a flare-up reaction might imply a different process from the primary elicitation of an allergic contact dermatitis. NGAL is primarily a protein of the neutrophil leukocyte, rarely observed in the flaring skin tissue (3), but NGAL may perhaps be released from neutrophil leukocytes activated in the circulation by TNF- α and/or IL-1.

Following this confirmation, clinically and biochemically, of our previous findings, the next question to be handled was that of a non-specific toxicity of the gold salt used for provocation. This could, however, be ruled out since no patients with nickel allergy provoked with gold (group 2) had any clinical reactions, rise of body temperature, or release of plasma cytokines. Vice versa, no clinical or biochemical reactions were observed when patients allergic to gold ingested a capsule of nickel sulfate (group 3). Clearly, patients with a contact allergy to gold do not seem to be non-specifically prone to eczematous flare-ups.

A further question to be answered was the response to the contact allergen in nickel allergy. As expected, clinical flare-up reactions were observed in 1/3 of the patients (group 1). The oral dose of 2.5 mg nickel regularly induces a flare-up in most patients with nickel allergy but the outcome is dependent, not only on dose, but also on time and intensity of previous patch tests (11). A rise of

body temperature was not registered in this group (Fig. 1). After systemic allergen challenge fever reactions have been observed in contact allergy to the "para group" (12) and to synergists (13), but never in nickel allergy (14, 15).

Most interestingly, there was a highly significant increase in plasma sTNF R1 after oral provocation in the nickel group (Fig. 2). The increase in plasma sTNF R1 indirectly indicates a release of TNF- α , although the plasma values for this cytokine were normal ($p = 0.26$). The receptor is released in larger amounts than TNF- α , reaches peak values later, and has a slower turnover.

Thus, the cytokine release induced by gold in patients with gold allergy has now also been demonstrated in patients allergic to nickel after nickel provocation, albeit implying just 1 of the cytokines assayed. The biochemical reaction might therefore be considered characteristic for the dynamics of the flare-up in contact allergy. Whether the cytokines released are instrumental in mediating the contact dermatitis flare-up, or a consequence thereof, remains to be demonstrated.

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