

## Flare-up of Allergic Contact Dermatitis in the Mouse after Topical Distant Provocation

HALVOR MÖLLER

*Departments of Dermatology and Experimental Research, Lund University, Malmö, Sweden*

Möller, H. Flare-up of allergic contact dermatitis in the mouse after topical distant provocation. *Acta Derm Venereol* (Stockh) 1984; 64: 125-128.

Flare-up of contact dermatitis after exposure to the antigen on another skin area was studied in the mouse. Animals were sensitized and challenged with picryl chloride. After healing, the ear dermatitis flared up following provocation on abdominal skin. Likewise, nickel-sensitized mice flared in one ear when exposed to the antigen on the other ear. This model could be of use in pathogenetic studies on secondary eruptions which occur so frequently in contact dermatitis. *Key words: Picryl chloride; Nickel; Secondary eruption.* (Received May 4, 1983.)

H. Möller, Department of Dermatology, General Hospital, S-21401 Malmö, Sweden.

Disseminated eczematous eruptions occur frequently during the course of allergic contact dermatitis. In many instances these eruptions do not appear as a consequence of antigen contact with the skin area in question. Instead, systemic exposure to the antigen, or a local spread from one skin site to another, have to be considered. In order to get a better understanding of the latter mechanism an attempt was made to design an experimental model for a distant flare-up after topical provocation. The experimental animal chosen was the regular laboratory mouse, so easily sensitized to potent antigens. The study comprised one such potent antigen, picryl chloride, but also nickel because of its great clinical importance.

### MATERIAL AND METHODS

*Animals.* Female NMRI albino mice were obtained from Anticimex AB, Stockholm, Sweden. Their weight was about 30 g, their age 2-3 months when starting the experiments.

*Drugs.* Picryl chloride was purchased from BDH, Poole, U.K.; before delivery, 20% water is added, thus reducing the figures given below to the same degree. Nickel sulphate p.a. was obtained from Merck, Darmstadt, BRD, and Polymyxin B sulphate from Pfizer, Brussels, Belgium. Polymyxin B was dissolved in physiologic saline and given in a dose of 10 mg/kg i.p. on 3 consecutive days.

*Picryl chloride experiments.* Sensitization (step I) was performed by a single painting onto a 3-4 cm<sup>2</sup> area of the shaved abdomen with picryl chloride 7% dispersed in 0.1 ml of 99.5% ethanol. The fluid was allowed to evaporate, after which the animal was not bandaged or otherwise restrained. One week later (step II) the animals were challenged by painting both ears with picryl chloride 0.5% in 0.05 ml olive oil. The resulting contact dermatitis (1) was allowed to recede for 1-8 weeks, usually 2 weeks, before starting topical provocation. This, step III, was performed by painting the abdomen with picryl chloride 0.5% in ethanol, followed by the same in olive oil. Controls were given the two vehicles only.

The animals were examined 24 h later (in some experiments at 4 or 72 h) for a flare-up of the previous ear dermatitis. Two parameters were used: erythema and edema. The presence or absence of erythema was determined by visual inspection by comparing entire animal groups as well as randomly assembled pairs from two groups. These comparisons were made "blind" by one observer. The degree of edema was determined after killing the animal and calculating the wet weight of ear tissue (2).

*Nickel experiments.* Sensitization was carried out by daily paintings for 3 weeks on the left ear with 20% nickel sulphate in methanol, as recently described (3). The animals were challenged 3 days after

Table I. Flare-up of ear contact dermatitis after provocation on abdomen

The difference in wet weight between groups 1 and 2 is 11% and highly significant ( $p < 0.001$ ). Treatment with Polymyxin B does not prevent the wet weight increase (no statistical difference between groups 1 and 3)

	Animal groups		
	1	2	3
Sensitization	+	+	+
Challenge	+	+	+
Treatment	Saline	Saline	Polymyxin B
Provocation	Picryl chloride	Vehicles	Picryl chloride
W.Wt. % $\pm$ SD	60.6 $\pm$ 2.0	54.6 $\pm$ 1.6	58.3 $\pm$ 3.2

the last exposure by painting the right ear with 0.5% nickel sulphate in methanol. The mice were sacrificed 24 h later and the wet weight of the left ears calculated.

*Calculation.* Groups of 8 mice were used. In the picryl chloride experiments the mean wet weight of both ears was taken for statistical comparisons (Student's *t*-test). In nickel experiments, values for wet weight of left ears were used for statistical analysis.

## RESULTS

*Picryl chloride.* According to the experimental design all animals were sensitized on the abdomen and challenged on the ears. When after a further 2 weeks they were again exposed to the antigen, now on the abdomen, the previous contact dermatitis of the ears flared up. This was established by the appearance of erythema and edema, not present in animals provoked with the vehicle only (Table I). The increase in ear tissue wet weight in different experiments was 3–12%.

In one experiment the time course of the flare-up was studied. The edema was maximal 24 h after topical provocation, but only weak and non-significant after 4 and 72 h. The erythema was evident at all three readings but maximal at 24 h (Table II). In a long-term experiment, provocation on the abdomen was attempted 1, 2, 4 and 8 weeks after challenge on the ears. A positive flare-up, evidenced as appearance of erythema as well as edema in comparison with control groups, was observed after all four periods.

Polymyxin B, given on the last 3 days before topical provocation, showed a slight preventive effect on the development of erythema and edema in some flare-up experiments. This was never statistically significant, however (Table I).

Table II. Flare-up of ear contact dermatitis after provocation on abdomen

Erythema was determined in mice provoked with picryl chloride (A) or with vehicles (B). See also Material and Methods

Time after provocation	Group examination	Pair examination
4 h	A redder than B	5 pairs: A redder than B 3 pairs: no difference
24 h	A redder than B	8 pairs: A redder than B
72 h	A slightly redder than B	3 pairs: B redder than A 2 pairs: no difference

*Nickel.* Two groups of mice, both sensitized on the left ear with nickel sulphate, were compared. One group was challenged with nickel/methanol on the right ear, the other with methanol only. At sacrifice 24 h later, the left ears of the nickel-challenged group had a higher wet weight than the control group (Table III), indicating a flare-up of contact dermatitis after topical provocation.

## DISCUSSION

The concept of flare-up in allergic contact dermatitis has been used for various phenomena. Thus, on systemic exposure to the appropriate antigen, accidentally or experimentally, the contact dermatitis present usually flares up (4, 5). Even a contact dermatitis active several years previously is usually reactivated. The first symptoms of such a flare-up usually appear about 4 hours after systemic administration of the antigen. In nickel allergy, the histology of the flare-up was that of an eczematous delayed-type reaction (6) and this held true for the reactivated pompholyx as well as the flaring nickel patch test. In the guinea pig the flare-up after systemic exposure has been demonstrated with arsphenamine (7, 8), with dinitrochlorobenzene (9) and with chromate (10), and in the mouse with picryl chloride (1).

Topical administration of the antigen can also induce a flare-up of a distant contact dermatitis. It happens now and then during the patch test procedure and can incorporate an active as well as a previous dermatitis in the patient. This phenomenon should not be confused with the so-called spontaneous flare-up after DNCB sensitization occurring after 10–20 days in the identical skin area (11). The true flare-up of a distant contact dermatitis after topical exposure is probably the explanation for many exacerbations in eczematous patients, such as the allergids or auto-sensitization occurring in 33% of patients with hypostatic eczema (12), and the secondary eruptions in 44% of patients with contact dermatitis (13), in nickel allergy in particular—about 75% (4).

This was the reason why an experimental model for the distant flare-up was sought and considered important. In the mouse, contact allergy to potent antigens is easily induced and the challenge reaction well quantitated (2). In the present study one potent antigen, picryl chloride, and one weak, nickel sulphate, were used for sensitization, challenge and flare-up. With picryl chloride, contact dermatitis on the ear, allowed to subside for 2 weeks (1), was provoked to flare up when the abdominal skin of the animal was exposed to the antigen. With nickel sulphate, one ear which had been subjected to sensitizing paintings with the antigen, was induced to flare up by provocation on the other ear.

The flare-up with nickel sulphate, although statistically significant, was weak, which is probably a consequence of the difficulty with which the mouse is sensitized to nickel and the low grade of sensitivity (3). Therefore, for future work, the mouse model with picryl chloride is recommended.

The flare-up reaction was evidenced by erythema as well as edema and both were

Table III. *Flare-up of contact dermatitis on one ear after challenge on the other*

The difference between the two groups of mice sensitized to nickel sulphate is significant ( $p < 0.01$ )

	Sensitized on left ear	Challenged on right ear	Wet weight of left ear (% and SD)
Group A	Nickel	Nickel	52.9±2.4
Group B	Nickel	Vehicle	48.4±1.2

maximal 24 h after topical provocation. This is in accordance with findings after systemic exposure in man as well as in the experimental animal. Indications of an inflammatory response were obvious, however, even after 4 h. Moreover, the involvement of immunologic reactions other than delayed allergy in the flare-up has been discussed (14, 5). It was therefore felt warranted to demonstrate or exclude a pathogenetic role of biogenic amines, e.g. histamine, in the reaction. Pretreatment with Polymyxin B which is supposed to remove 53% of the histamine and 38% of the serotonin content of the mouse ear (15), did not, however, significantly influence the flare-up. A partial role of histamine or other inflammatory mediator cannot of course be excluded by this crude method.

In the guinea pig sensitized to DNCB or oxazolone, challenge on two different skin sites, one virgin and one healed after a previous challenge, resulted in a more intense dermatitis in this latter skin than in the virgin area (16). This could be explained by a local memory, activated in turn by retention of hapten-specific T lymphocytes. Of course this may also hold true for a flare-up after distant provocation as in the present work.

## ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Work Environment Found, grant 81-0099, and the Alfred Österlund Foundation. Mrs K. Lundberg gave skilful technical assistance.

## REFERENCES

1. Dahlbäck K, Möller H. Flare-up of contact dermatitis to picryl chloride in the mouse. *Acta Derm Venereol (Stockh)* 1981; 61: 342-344.
2. Möller H. Allergic contact dermatitis of the mouse ear. *Acta Derm Venereol (Stockh)* 1981; 61: 1-6.
3. Möller H. Attempts to induce contact allergy to nickel in the mouse. *Contact Dermatitis*. In press.
4. Christensen OB. Nickel allergy and hand eczema in females. A clinical and pathogenetic study with special references to the importance of ingested nickel. *Mal nö* 1981.
5. Menné T, Hjorth N. Reactions from systemic exposure to contact allergens. *Seminars in Dermatology* 1982; 1: 15-24.
6. Christensen OB, Lindström C, Löfberg H, Möller H. Micromorphology and specificity of orally induced flare-up reactions in nickel-sensitive patients. *Acta Derm Venereol (Stockh)* 1981; 61: 505-510.
7. Sulzberger MB. Hypersensitiveness to arsphenamine in guinea-pigs. I. Experiments in prevention and in desensitization. *Arch Dermatol Syphilol (Chicago)* 1929; 20: 669-697.
8. Kaplun BJ, Moreinis JM. Versuch der experimentellen Sensibilisierung gegen Salvarsan, bei Menschen und Tieren. *Acta Derm Venereol (Stockh)* 1930; 11: 295-304.
9. Frey JR, de Weck AL, Geleick H. Inhibition of the contact reaction to dinitrochlorobenzene by intravenous injection of dinitrobenzene sulfonate in guinea pigs sensitized to dinitrochlorobenzene. *J Invest Dermatol* 1964; 42: 189-196.
10. Polák L, Turk JL. Studies on the effect of systemic administration of sensitizers in guinea-pigs with contact sensitivity to inorganic metal compounds. I. The induction of immunological unresponsiveness in already sensitized animals. *Clin Exp Immunol* 1968; 3: 245-251.
11. Skog E. Spontaneous flare-up reactions induced by different amounts of 1,3 dinitro-4-chlorobenzene. *Acta Derm Venereol (Stockh)* 1966; 46: 386-395.
12. Haxthausen H. Generalized "Ids" ("Autosensitization") in varicose eczemas. *Acta Derm Venereol (Stockh)* 1955; 35: 271-280.
13. Zuber M. Untersuchungen über die Topographie der Ausbreitung und Streuung beim Ekzem. *Diss. an der Derm. Univ.-Klinik, Zürich* 1949.
14. Polak L, Frey JR, Turk JL. Studies on the effect of systemic administration of sensitizers to guinea-pigs with contact sensitivity to inorganic metal compounds. IV. Studies on the mechanism of the 'flare-up' reaction. *Clin Exp Immunol* 1970; 7: 739-744.
15. West GB. Comparison of the release of histamine and 5-hydroxytryptamine from tissues of the rat, mouse and hamster. *Int Arch Allergy* 1958; 13: 336-347.
16. Scheper RJ, von Blomberg M, Boerrigter GH, Bruynzeel D, van Dinther A, Vos A. Induction of immunological memory in the skin. Role of local T cell retention. *Clin Exp Immunol* 1983; 51: 141-148.