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Delayed Hypersensitivity Reactions Following Allergic and Irritant Inflammation

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Delayed hypersensitivity retest reaction 3 and 6 weeks after induction of allergic and irritant inflammation, was studied in 13 females with known hypersensitivity to nickel. An increased retest reaction compared to controls was observed only in sites of earlier specific allergic inflammation. Also a general down-regulation of the degree of hypersensitivity was observed at retesting. ***Key words: Retest reaction, Contact hypersensitivity, Nickel allergy.***

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In a study in patients with tuberculin hypersensitivity Weiss (1) demonstrated accelerated reactivity at retest sites as late as 240 days after the initial test. This phenomenon of accelerated and increased reaction at retest sites of delayed hypersensitivity has been investigated in guinea pigs (2, 3). In an extensive study by Scheper et al. (3) the time course and local persistence of T-lymphocytes in the retest reaction and the duration of local skin memory, respectively, was investigated. In this latter study it was shown that testing of non-specific inflammation (toxic-irritant) sites also resulted in a significantly increased retest reaction and that this phenomenon persisted for at least 5 weeks. In the following study we have investigated retest reactions following allergic and non-allergic inflammation in a human model.

MATERIALS AND METHODS

Subjects

Thirteen female volunteers, mean age 35.4 years (range 24–53 years) sensitive to nickel were enrolled in the study after approval by the Ethics Committee at Lund University. Hypersensitivity to nickel had previously been demonstrated by patch testing with ICDRG standard series including nickel sulphate ($NiSO_4$) 5% in petrolatum (Chemotechnique Diagnostics, Malmö, Sweden) in Finn Chamber (Epitest LTD, Finland).

Screening of the degree of hypersensitivity to nickel

Initially each subject was tested with 3 concentrations of nickel sulphate ($NiSO_4$, $6H_20$), 20 µl of 2.4%, 0.6%, and 0.15%. The nickel solution was micropipetted into filter paper discs of small Finn Chambers (r 8 mm) on Scanpor and immediately placed on the lateral aspect of the inner left forearm. The patches were removed after 48 h and reading was performed 24h later. The reactions were scored:

0 = No reaction,
+ = Erythema and infiltration,
++ = Erythema, infiltration and papules,
+++ = Erythema, infiltation, papules and vesicles/exsudation.

Induction of allergic and irritant inflammation

At the time of test reading irritant as well as allergic inflammation was induced on the inner aspect of both forearms, avoiding the areas used for screening of hypersensitivity to nickel on the left forearm. This procedure was caried out by our laboratory technician in a randomized order.

To induce irritant inflammation two different substances were used. Sodium lauryl sulphate 3% 20 µl was micropipetted into filter paper disks of small Finn Chambers on Scanpor. Also a fixed amount of dithranol 1% (Micanol®, Hydro Pharma AB, Sweden) was placed in Finn Chambers. To induce allergic inflammation 20 µl of 2.4% nickel sulphate was micropipetted into small Finn Chambers. The test solutions (sodium lauryl sulphate and nickel sulphate) were removed after 48 h and the test reactions read 24 h later. The dithranol was removed after 1.5 h and the test reaction read at the same time as above.

Rechallenge

Three and 6 weeks later the subjects were retested on all earlier induced inflammation sites and a control site with 20 µl of the lowest concentration of nickel sulphate which in the primary screening test produced a ++ reaction. Application time and time for test reading was the same as earlier mentioned. Until rechallenge the subjects kept the induced inflammation sites visible with a skin marker, enabling us to rechallenge on the exact area of the former inflammation. The rechallenge test reaction was scored as mentioned above.

Statistical significance was tested with the Wilcoxon rank sum test.

RESULTS

The initial allergic and irritant testing produced in general the same degree of inflammation.

In Table I the evaluation of the eczematous reaction of the rechallenge on all sites of earlier induced inflammation and a control site after three and six weeks is presented. A significant difference between the sums at the nickel sulphate site and the control site after 3 ($p = 0.007$) and 6 weeks ($p = < 0.005$), respectively, was observed. There was no significant difference beween the control and dithranol or sodium lauryl sulphate sites, neither after 3 nor 6 weeks.

Also the intensity of the control test reactions at 3 as well as 6 weeks reading was significantly weaker ($p = 0.0025$, 3 weeks and $p = 0.0025$, 6 weeks) than the initial ++ reaction chosen as the reference value.

A lower, however not significant intensity of the specific retest reaction was observed after 6 weeks when compared to the reaction at 3 weeks.

DISCUSSION

In this study an increased retest reaction compared to controls was observed 3 and 6 weeks after induction of inflammation, corresponding only to the specific allergic inflammation site and not to sites of earlier irritant inflammation. This is in contrast to the results by Scheper et al. (3) in which an increased retest reaction was observed on specific as well as non-specific inflammation sites at least up to 5 weeks following induction of inflammation. The results observed in the present human model correspond to the results published earlier in

Table I. *The eczematous reaction of the rechallenge in sites of earlier induced inflammation and a control site after 3 and 6 weeks.*

Patient	3 weeks, right arm				6 weeks, left arm			
	Ni	D	SLS	Control	Ni	D	SLS	Control
1	+++	+	+++	+	++	++	+++	++
2	+++	+++	+++	++	+	+++	++	+
3	+++	+++	−	++	++	++	−	++
4	++	+	+++	+	++	++	++	++
5	+	−	++	−	−	+	−	−
6	+++	+++	++	++	++	+	+	−
7	++	−	+	+	+	+	+	+
8	+++	++	+	+	++	+	+	−
9	++	−	−	+	++	−	++	+
10	++	+	+	+	++	−	+	+
11	++	+	+	+	+++	+	−	+
12	++	+	+	+	++	++	+	−
13	+++	−	++	+	+++	−	++	+
Sum	31	16	20	15	24	16	16	11

Ni = Nickel sulphate; D = Dithranol; SLS = Sodium lauryl sulphate; +++ = 3; ++ = 2; + = 1.

which flare-up reactions after oral provocation with allergen were highly specific to previous allergic sites and did not show any reaction to previous irritant reaction site (4). From these studies we may conclude that the specific retest reaction and the flare-up reaction in principal is similar in nature.

In this study the increased retest reaction could be demonstrated for at least 6 weeks. Following oral challenge with nickel flare-up reactions can appear years after an earlier inflammation (5). In the study by Scheper et al. (3) in guinea pigs the ability of increased retest reactions persisted longer at the specific inflammation sites than at the non-specific sites. This speaks in favour of the increased specific retest reaction being a long-lasting phenomenon.

Obviously, the memory function is present in the skin for a long time following a specific inflammation. As shown by Scheper et al. (3) hapten specific T-lymphocytes may persist for several months in previous sites of inflammation, indicating that these cells have a memory function and play an important role mediating the increased reaction.

In a study by Christensen et al. (6) small scattered perivascular cell infiltrates consisting of macrophages, mast cells, T-lymphocytes and Langerhans' cells were found in 6–8 weeks old patch test sites. In another human model of another type of flare up reaction (fixed erythema) we have described the location of T-cytotoxic suppressor lymphocytes in reaction sites three weeks after drug challenge (7). These studies speak in favour of T-lymphocytes having a memory function in human skin. The exact phenotype of these T lymphocytes is to our knowledge still unknown.

Immunohistochemical investigations of flare up reactions have shown a predominant amount of T-helper lymphocytes in the dermal and epiderml infiltrates (6, 8). This is probably a secondary phenomenon following release of cytokines from hapten-specific memory cells.

Besides the local increased specific retest reaction we also observed a significant general downregulation of the test reaction expressed by a lower intensity of the control test reaction than expected compared to the reference value. The same phenomenon has been described in humans by Thestrup Pedersen (9) and in guinea pigs by Blomberg et al. (10). Apparently, a balance between up- and downregulation exist in allergic contact dermatitis and needs further investigation with regard to time intervals and mechanisms involved.

Does the increased local reactivity bear any clinical relevance? At least the necessity of allergen elimination to avoid flare up by a relatively small amount of allergen might be of importance.

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