

Histological distinction between early allergic and irritant patch test reactions: follicular spongiosis may be characteristic of early allergic contact dermatitis

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Comparative light microscopic studies have revealed subtle differences between allergic and irritant reactions in the skin. In the search for specific differences, we focussed on the early inflammatory response. This pilot study was conducted to test the hypothesis that follicular spongiosis can differentiate between early allergic and irritant patch test reactions. 8 patients with known contact allergy to either colophony or quaternium-15 participated in the study. In each patient, allergic and irritant patch tests reactions were elicited, and 4-mm punch biopsies were taken after 6–8 h from clinically equipotent reactions. Paired sets of slides were assessed blindly by 2 pathologists. 1 patient showing a pityrosporum folliculitis was excluded from the study. All biopsies from allergic patch tests were characterized by follicular spongiosis, while biopsies from irritant patch tests showed no recognizable changes except a slight follicular spongiosis in 1 patient. The 2 pathologists agreed independently on the correct classification in 6 out of 7 cases ($p=0.0156$). We tested an optimized model, selecting non-irritant allergens and a well-known irritant. Further investigations are needed to elucidate the diagnostic significance of the histological classification of allergic and irritant cutaneous reactions in punch biopsies.

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The evaluation of a positive patch test is based on the reaction pattern combined with clinical information and knowledge about the patch test material. In several cases, it is difficult to differentiate between allergic and irritant test reactions. Various re-test procedures may be applied to circumvent this problem. In doubtful cases, histological investigations are of little diagnostic help (1). Despite the different pathogenesis, allergic and irritant reactions show remarkable similarity with respect to histology and immunohistology (2–5). In the fully-developed allergic and irritant patch test reaction, the composition of the inflammatory cellular infiltrates is identical. The lack of specific changes has been explained by the existence of a common inflammatory pathogenic pathway. According to this theory, different initiating events

are able to activate a common amplification mechanism in the skin (6). The inflammatory response is considered a stereotypic process and the accumulation and activation of T-cells take place independent of specific sensitivity (7). Recent studies support the possibility that contact allergens and irritants may stimulate different patterns of epidermal cytokine production (8).

Comparative light microscopic studies have revealed subtle qualitative and quantitative differences between allergic and irritant reactions (9–14). In a study characterizing the early cellular responses in allergic and irritant contact dermatitis, the first light microscopic changes were observed at 4–8 h in the allergic patch test reaction, compared with 8 h in the irritant reaction (15).

Light microscopy has so far proved unsuccessful

Table 1. Histologic grading of irritant and allergic patch test reactions^{a)}

	Follicular spongiosis (n=7)	Exocytosis (n=7)	Interfollicular spongiosis (n=7)
allergic patch tests			
grade 0	—	1	5
1	4	4	2
2	3	2	—
3	—	—	—
irritant patch tests			
grade 0	7	6	7
1	—	1	—
2	—	—	—
3	—	—	—

^{a)} The grading is shown for the pathologist who diagnosed the reactions correctly.

in distinguishing between allergic and irritant patch test reactions. Looking in the right place at the right time might, however, reveal more characteristic differences. This pilot study was undertaken to test the hypothesis that early follicular spongiosis may differentiate between allergic and irritant reactions.

Materials and Methods

8 individuals, all women aged 31–51 (mean 39) years, with known contact allergy to either colophony (5 patients) or quaternium-15 (3 patients), participated in the study after giving informed consent. The study was approved by the local ethical committee. These 2 allergens were chosen because they rarely give rise to non-specific or irritant reactions. None of the patients included had a personal history of atopy or had received immunosuppressive or UV-treatment. In each patient, 1 patch test with the relevant allergen (TRUE Test™, Alk-Abello, Hørsholm, Denmark) and a dilution series of the irritant (benzalkonium chloride 0.2% aq., 0.5% aq. and 1.0% aq., Finn Chambers®, Epitest OY, Tuusula, Finland) were applied in parallel duplicates on the upper back, where follicles are more abundant than on the lower back.

Patch tests on one side of the back were removed after 6 h (3 patients) or 8 h (5 patients) and the results were read 30 min later. 2, 4 mm punch biopsies were taken from each patient, 1 from the allergic patch test site and 1 from an irritant patch test site. We attempted to take biopsies from the 1 of the 3 irritant reactions giving clinically equipotent inflammation as compared to the allergic reaction. When the irritant patch test gave no visible reaction at 6–8 h, the patch test with the strongest concentration of benzalkonium chloride was

chosen for biopsy. The identical patch tests on the other side of the back were removed after day (D) 2 and read according to ICDRG standard technique at D 3 and D 7.

The biopsy specimens were fixed in formalin, embedded in paraffin and serially sectioned, and stained with hematoxylin and eosin to visualize representative follicles. The slides were assessed blindly by 2 pathologists using semiquantitative assessment of the following parameters: follicular spongiosis, exocytosis of inflammatory cells into the follicular epithelium and interfollicular spongiosis. Looking at paired sets of slides from each patient, one was classified as allergic and one as irritant, based on the presence or absence of follicular spongiosis, respectively. For statistical analysis, the Sign test was applied.

Results

In 1 patient, a slight pityrosporum folliculitis blurred the histopathological assessment, and this patient was subsequently excluded from the study. Among the remaining 7 patients, the 2 pathologists agreed independently on the correct classification in 6 cases of either irritant or allergic patch test reactions. The results were of statistical significance, $p=0.0156$.

Colophony was tested in 5 patients. Patch test reactions read at 6–8 h revealed 3 doubtful positive reactions and in 1 of the 2 remaining patients a follicular reaction was described. Quaternium-15 patch tests showed no clinical reactions when read at 6–8 h. All allergic patch tests were positive at D 3 and D7.

The early reading of irritant patch tests revealed a visible reaction in only 1 patient at both 0.5% and 1.0% concentrations. In this patient, the bi-

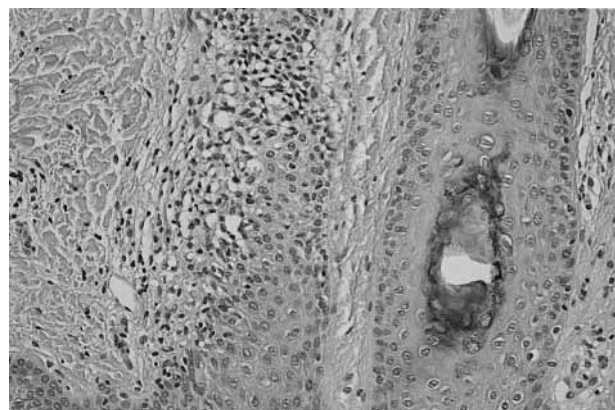


Fig. 1. Punch biopsy from a patch test site (colophony, 8 h) showing follicular lymphocytic exocytosis and follicular spongiosis. The interfollicular epithelium showed no changes (not shown).

opsy was taken from benzalkonium chloride 0.2%, while the remaining patients were biopsied from the 1.0% patch test sites. Benzalkonium chloride 0.5% and 1.0% aq. in most patients gave bullous reactions at D 3 and D 7 readings.

Biopsies from the irritant patch test reactions showed no recognizable changes except a follicular exocytosis in 1 patient. In this patient, a visible reaction to the irritant was observed at the early reading as mentioned above. The 2 pathologists disagreed on the classification of the biopsies from the patient.

The readings of the pathologist who classified the reactions correctly are shown in Table 1. All biopsies obtained from the allergic patch test reactions showed follicular spongiosis (Fig. 1). The light microscopic intensity of the reaction seemed to be related to the time of allergen exposure. Allergic reactions scoring grade 2 spongiosis in the follicles were all found in patients having the patch tests removed after 8 h, and the scoring of exocytosis also increased with the time of allergen exposure. All but 1 biopsy from the allergic patch test sites showed lymphocytes in the follicular epithelium. Where this was not observed, the overall histopathological changes were sparse. Eosinophilic and polymorphonuclear leukocytic exocytosis was noticed in biopsies from irritant as well as allergic patch test reactions, but only occasionally and was not graded. Only rarely was additional spongiosis observed in the interfollicular epithelium.

Discussion

In the present study, we were able to distinguish histologically between strong allergic and strong irritant patch test reactions. The observed specific differences were an early spongiosis and lymphocytic exocytosis of the follicular infundibulum in the allergic reaction, while hardly any changes were seen in the irritant reaction. The blinded histopathological evaluation by the 2 independent observers was consistent in all but 1 case. In this pilot study, however, we chose an optimized model to decide if it was at all possible to distinguish between allergic and irritant reactions. In selecting non-irritant allergens and a well-known irritant (16), mixed reactions were avoided. To test the hypothesis further, more allergens and irritants should be evaluated. The lack of equipotency of the reactions or differences in the time course of the 2 reaction types rather than qualitative differences could be responsible for the differences observed. The timing of biopsy seems to be critical and changing it by only 2 h is reflected in the intensity of the morphological changes. Sequential

biopsies as well as biopsies from a dilution series of allergen and irritant could elucidate this further. Moreover, randomization of slides rather than paired observations should be done.

The observed histological differences are in accordance with more recent studies suggesting that qualitative differences do occur in the inflammatory responses induced by an allergen and an irritant, respectively. To elucidate the significance of these results, further investigations are needed. The findings are of theoretical interest and do not at present provide a diagnostic tool in the differentiation between allergic and irritant test reactions.

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