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Allergic and Irritant Contact Responses to DNFB in BN and LEW Rat Strains with Different T_H1/T_H2 Profiles

MICHAEL J. PESZKOWSKI, GUNNAR WARFVINGE and ÅKE LARSSON

Department of Oral Pathology, Centre for Oral Health Sciences, Lund University, Malmö, Sweden

BN and LEW rats possess different and extreme profiles of T_H1/T_H2 cells. The main objective of this study was to determine if this constitutively expressed property may influence the propensity to develop contact allergy to a potent contact sensitizer, 2,4-dinitro-1-fluorobenzene (DNFB). In order to avoid overlapping reactions due to toxicity, we defined non-irritant threshold levels of DNFB histologically in ear skin, dorsal skin and oral mucosa prior to the sensitization experiments. Evaluation of the elicitation response was carried out by an invasive method (biopsy). LEW rats proved to express a more extensive challenge response to DNFB than rats of the BN strain. Further, epicutaneous sensitization with DNFB gave a better challenge response than the subcutaneous route regardless of rat strain. In ear skin, but not in oral mucosa, the response was more vigorous after five than after two exposures. Our results are discussed with respect to the possibility that the T_H1 type cells may play a significant role in the development of experimental contact allergy. ***Key words: contact allergy; T-lymphocytes; hypersensitivity.***

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M. J. Peszkowski, Department of Oral Pathology, Centre for Oral Health Sciences, University of Lund, S-214 21 Malmö, Sweden.

The etiology and pathogenesis of oral mucosal lesions referred to as adverse reactions to dental materials remain obscure, but they have been claimed to represent toxic, contact allergic or autoimmune reactions (1). The HLA haplotype seems to influence expression of such lesions (2), supporting the concept of contact allergy or autoimmunity. In previous studies, we have examined some aspects of oral contact allergy in a model utilizing outbred SD rats (3–6). However, different animal strains are known to be differently disposed to T-cell-mediated contact allergy. Certain mouse and guinea pig strains, empirically found to be good responders, have therefore been preferred in commercial predictive screening tests for potential sensitizers (7). Although it is obvious that responsiveness may be correlated to MHC class II haplotype (2, 8), the exact nature of these differences is as yet unknown. In recent years, it has become evident that T-cell responses may follow different pathways depending on whether T_H1 cells producing IL2/IFNγ or T_H2 cells producing IL4–6 dominate the response to a particular antigen (9, 10). BN and LEW rats possess different and extreme profiles of resting T_H1 and T_H2 cells (11), which seem to influence or reflect their respective propensity to react upon various antigenic stimuli. Thus, BN rats [$T_H2:T_H1 \approx 27$; (11)] develop a supposedly T_H2-mediated autoimmune syndrome upon Hg administration (12), whereas Hg-exposed LEW [$T_H2:T_H1 \approx 0.9$; (11)] activate $CD8^+$ suppressor cells leading to resistance (13). In contrast, retinal antigen-exposed LEW develop experimental autoimmune uveoretinitis, a T_H1-dependent disease to which BN are resistant (14, 15). Contact allergy seems to be mediated mainly via the T_H1 pathway emanating from CTL activation (9). The propensity of an individual to respond in a T_H1-biased fashion to a particular antigen may thus decide if contact allergy develops or not. Whether this property is reflected by the constitutive T_H1/T_H2 profile of the individual is, however, not known. A main objective of this study has been to test this hypothesis in the rat.

A plethora of test methods are available in the field of contact allergy research, some of which evaluate the primary immune response in lymph nodes (16). However, we believe the effector phase is a better indicator of T_H1/T_H2 differentiation than the primary response, since the cellular activity of the challenge reaction reflects mainly the strength of the T_H1 limb taking into consideration a possibly differentiated migration pattern among T-cell subsets (9). In the skin, contact allergy is usually assessed by scoring the challenge-induced erythema and/or oedema (16). However, such strategies are not easily applicable to the oral mucosa (5). Furthermore, it may be argued that non-invasive assays measure mainly vascular parameters, whereas the predominantly T cell mediated tissue response characteristic of contact lesions (6) cannot be accurately studied with these methods. Thus, evaluation of the cellular response requires invasive techniques and importantly, it also requires the test substances to be used at non-irritant concentrations in the elicitation phase in order to avoid overlapping reactions due to toxicity (5).

The purpose of the present study was to establish a standardized test protocol for a potent contact sensitizer (2,4-dinitro-1-fluorobenzene, DNFB) in rat oral mucosa and skin. We have defined the irritant threshold levels of DNFB histologically in order to determine the relevant duration and dosage in each of three different rat strains. Subsequently, an effort was made to reveal any inter-strain differences between BN and LEW with regard to the intensity of the contact allergic response.

MATERIAL AND METHODS

Animals

Outbred SD rats, inbred BN and LEW rats, both sexes, weighing 250–400 g, were used. The SD and LEW strains were obtained from Møllegaard Breeding Center, Skensved, Denmark, and the BN strain from the Wallenberg Laboratory, Lund, Sweden. Animals were kept in separate cages on standard diet with tap water *ad libitum*.

Neuroleptanalgesia

Etorphine-acepromazine (Immobilon®; 12.5 µg/kg body weight) and its antidote diprenorphine (Revivon®; 45 µg/kg body weight) were used.

Chemicals and solutions

DNFB (Sigma Chem. Co.), used for epicutaneous and epimucosal application, was dissolved in acetone/olive oil (4:1). The solution used for injections was prepared by diluting a stock solution of DNFB in absolute ethanol, with saline, yielding a final ethanol concentration of 4%.

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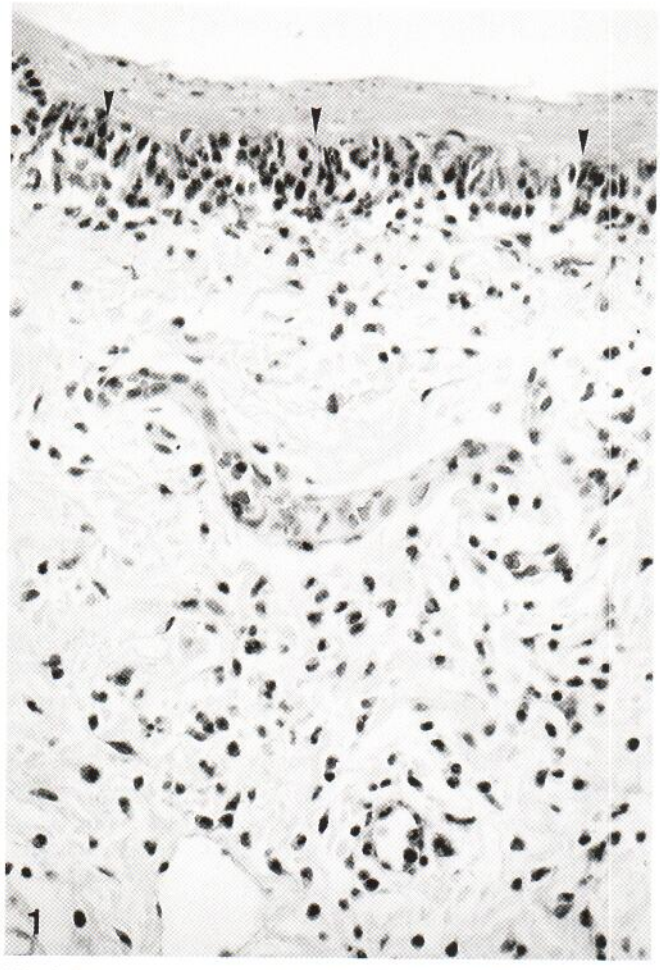

Fig. 1. Rat oral mucosa, 6 h after treatment with irritant (0.2%) DNFB solution in acetone/olive oil, showing massive, PMN-dominated inflammatory infiltrate, also encroaching upon surface epithelium (*arrowheads*). × 400.

Experimental protocol

Irritant threshold levels. Initially SD rats were used. The dorsal skin was shaved the day before application of test solution, the buccal mucosa was tested as described elsewhere (3), and the dorsal central part of the ear was also used. Twenty-five μl (skin) or 10 μl (oral mucosa and ear) of test solution was applied with a micro pipette to the test site during 1 min under neuroleptanalgesia. Challenged tissues were excised 6 h later, processed for routine histology and examined in a light microscope. A reaction was regarded as irritant when surface necrosis was present and/or inflammatory cells (PMN) could be clearly identified outside blood vessels (Fig. 1).

Based on the results obtained in SD rats, we repeated our study in BN and LEW rats in order to confirm irritant threshold levels in these strains, and as a way of determining elicitation protocols (see below).

Sensitization and elicitation. BN ($n = 14$) and LEW rats ($n = 14$) were used. Animals were sensitized by two or five daily applications of 60 μl of 0.5% (450 μg) DNFB on different sites of the shaved dorsal skin. Alternatively, sensitization was performed by two or five injections daily of 0.3 ml 0.025% (115 μg) DNFB subcutaneously (dorsal skin). Elicitation was carried out 5 days later in the left buccal mucosa and on the dorsum of the left ear with 10 μl 0.02% (3 μg) DNFB in acetone/olive oil. Animals were sacrificed 24 h after elicitation. Samples of challenged buccal mucosa and ear were excised and processed for routine histology.

Table I. *Irritant reaction to topically applied DNFB in acetone/olive oil, in rat tissues*

Rat strain	Concentration	Ear skin	Dorsal skin	Oral mucosa
SD	0.25 %	6/6[a]	5/5	2/2
	0.2 %	nd[b]	4/4	3/5
	0.1 %	4/5	5/7	1/7
	0.05 %	1/6	0/7	0/7
	0.025 %	1/6	nd	nd
BN	0.1 %	4/6	6/6	3/6
	0.02 %	1/6	1/6	0/6
LEW	0.1 %	6/6	5/6	2/6
	0.02 %	1/6	0/6	1/6

[a] number of positive animals/number of animals tested.
[b] nd = not determined.

Statistical analysis

Scoring of elicitation responses in ear skin and oral mucosa was performed independently by the three authors and in a blind fashion, assessing the changes according to the following semiquantitative scale:

0 = none or doubtful increase in the number of inflammatory (mononuclear) cells; 1 = weak reaction, minor but indisputable increase in the number of inflammatory (mononuclear) cells; 2 = moderate inflamma-

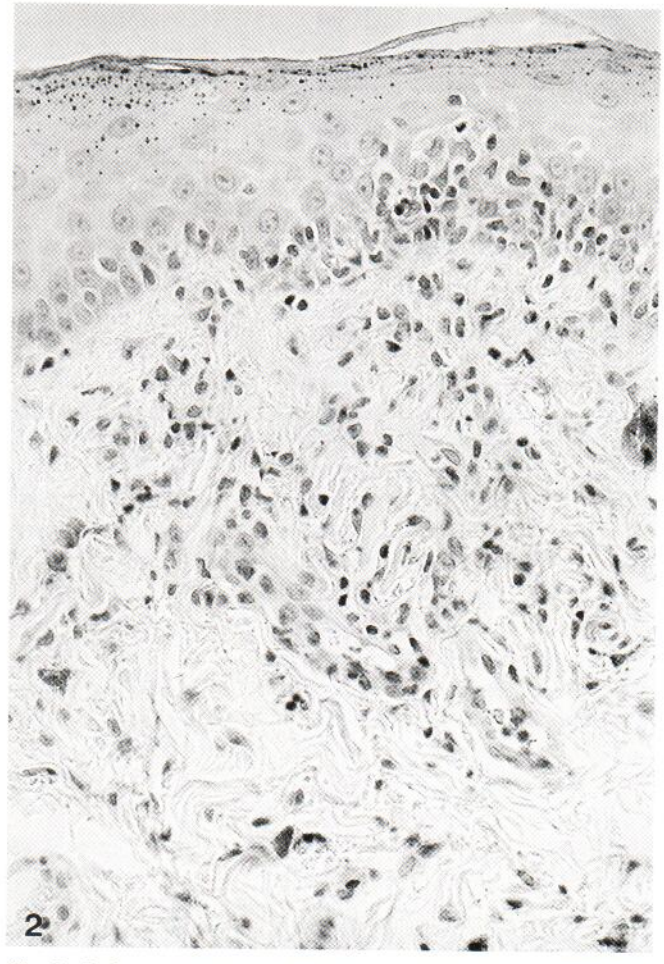

Fig. 2. Oral mucosa of pre-sensitized rat, 24 h after challenge with non-irritant (0.02%) DNFB solution, showing a moderate (score 2) mononuclear inflammatory infiltrate. × 400.

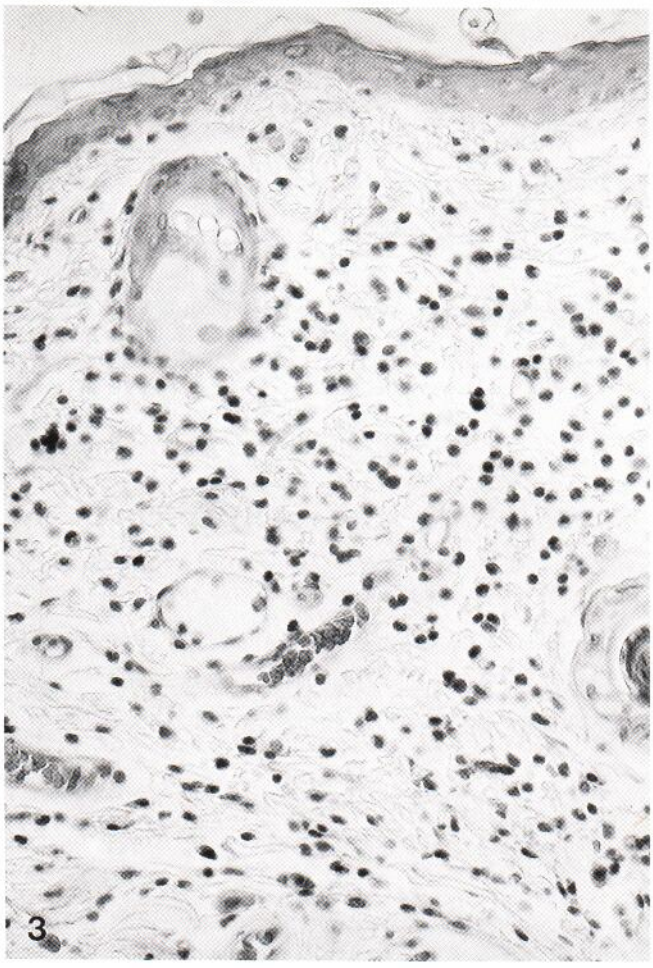

Fig. 3. Ear skin of pre-sensitized rat, 24 h after challenge with non-irritant (0.02%) DNFB solution, showing a strong elicitation response, with an inflammatory infiltrate composed predominantly of mononuclear cells. × 400.

tory reaction; 3 = strong inflammatory reaction. Each specimen was assigned the mean value of the three scores (observers), and statistical analysis was performed with the Mann-Whitney test and the paired rank sum test.

RESULTS

Irritant threshold level

Table I shows the reactions to different concentrations of topically applied DNFB in ear skin, dorsal skin and oral mucosa. Untreated (apart from shaving), and acetone/olive oil-treated control animals displayed a limited number of PMN in blood vessels only. Thus, such cells were accepted as a normal finding and not recorded in the test animals. Based on the results obtained in SD rats, LEW and BN rats were treated with 0.02% DNFB (non-irritant concentration in SD) and 0.1% DNFB (lowest irritant concentration in SD). There were no significant inter-strain differences between the irritant reactions of SD, LEW and BN rats.

Sensitization and elicitation

Oral mucosa in sensitized animals, challenged with a non-irritant DNFB solution, showed an almost purely mononuclear cell infiltrate (Fig. 2) as contrasted to the PMN-dominated irritant responses (Fig. 1). In challenged ear skin, the inflammatory infiltrates comprised predominantly mononuclear cells with a minor population of PMN (Fig. 3).

Table II. *Challenge response following different routes of sensitization with DNFB in BN and LEW rats*

Rat strain	Challenge site	Sensitization route		
		Epicutaneous	Subcutaneous	Mean
LEW (n = 14)	Ear skin	2.0±0.8[a]	1.0±0.8	1.5±0.9
	Oral mucosa	1.5±1.0	1.0±0.5	1.2±0.7
	Mean	1.7±0.4 (n = 7)	1.0±0.5 (n = 7)	1.3±0.6 (n = 14)
BN (n = 14)	Ear skin	1.6±1.2	0.7±0.4	1.2±1.0
	Oral mucosa	1.1±0.9	0.6±0.4	0.9±0.7
	Mean	1.4±0.6 (n = 7)	0.7±0.4 (n = 7)	1.0±0.6 (n = 14)

[a] mean value (± SD) of the mean scores assigned by 3 independent observes.

LEW rats were found to be more easily sensitized by DNFB than were BN rats ($p < 0.03$). This difference was statistically significant when all animals were included, but not within any one of the subgroups (Table II).

The elicitation response in ear skin was often stronger than in oral mucosa, although the difference was not statistically significant when all animals were included (Table III). However, there was a clear difference between ear skin and oral mucosal responses in animals that were sensitized five times ($p < 0.01$). Also, this group showed slightly stronger challenge responses than animals treated with DNFB twice ($p < 0.06$). Sensitization was more effective via the epicutaneous route than via subcutaneous injections when all animals were included ($p < 0.01$; Table II).

DISCUSSION

The main objective of this study was to compare two rat strains, known to exhibit different and extreme T_H1/T_H2 profiles (11), with regard to their propensity to develop contact hypersensitivity. In order to assess this property, it was essential to avoid

Table III. *Challenge response in ear skin and oral mucosa following sensitization with different numbers of DNFB applications in BN and LEW rats*

Rat strain	Challenge site	Number of DNFB applications		
		2	5	Mean
LEW (n = 14)	Ear skin	1.3±1.1[a]	1.8±0.6	1.5±0.9
	Oral mucosa	1.2±0.8	1.2±0.5	1.2±0.7
	Mean	1.2±0.6 (n = 8)	1.5±0.5 (n = 6)	1.3±0.6 (n = 14)
BN (n = 14)	Ear skin	0.7±0.5	1.7±1.2	1.2±1.0
	Oral mucosa	1.2±0.8	0.5±0.3	0.9±0.7
	Mean	1.0±0.6 (n = 8)	1.1±0.7 (n = 6)	1.0±0.6 (n = 14)

[a] mean value (± SD) of the mean scores assigned by 3 independent observers.

overlapping irritant effects of the allergen at elicitation. In the literature, allergic and irritant contact reactions are considered to be (immuno-) histologically similar (17, 18), but our own experience is that these types of reactions may be readily differentiated in their early stages (4–6). Consequently, we have found that irritancy is best evaluated at 6 h whereas elicitation responses in sensitized animals have been assessed 24 h after challenge. From our findings of irritant threshold levels in SD, BN and LEW rats we conclude that the irritant effect of DNFB is a stereotype, strain-independent response. Occasionally, scarce PMN were seen outside blood vessels after challenge with DNFB at the "non-irritant" concentration, but never an influx of mononuclear cells.

We found that oral mucosa was slightly less sensitive to challenge than skin, especially ear skin. This was most likely due to the better retention of DNFB in skin, as the oral mucosa is protected by a saliva film which contributes to the clearance of the test substance (19).

Sensitization was performed by the application of test substance either twice or five times, the latter giving more effective sensitization. Compared to Kurimoto & Streilein (20), we have used high sensitization doses in order to attain strong sensitization, but in order to assure non-irritancy, our elicitation concentration falls below theirs by a factor 10. In contrast to Kurimoto & Streilein (20) we were not able to sensitize efficiently with intradermal injections, where only weak reactions were seen (Table II). It must, however, be kept in mind that we have assessed different aspects of the hypersensitivity reaction (cellular vs. vascular). Since DNFB-specific IgE-production has been reported (21), IgE may possibly influence the macroscopic scoring of oedema. Alternatively, the reason for our failure may be that the dose of sensitizer was too high, resulting in tolerance (22, 23). We do not believe that the amount of antigen used for sensitization was too low since it exceeded the amount used by Kurimoto & Streilein (20), and it was also in the range of the dose we applied by the epicutaneous route.

Most individuals and experimental animals, including SD, LEW and BN rats, may be sensitized by strong contact allergens like DNFB, whereas some animal strains are more prone than others to develop contact allergy to weak and moderate sensitizers (7). By histologically assessing the intensity of the challenge reaction, we found LEW to be more easily sensitized by DNFB than were BN. Contact allergy involves primarily the cellular arm of the immune response, which is believed to be T_H1-regulated (9). Consequently, although no reliable immunohistochemical markers are available to differentiate between activated T_H1 and T_H2 cells in situ, there is reason to believe that the T_H1 disposition of the $CD4^+$ population in LEW rats is responsible for the increased reactivity.

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