Influence of Epidermal Permeability Barrier Disruption and Langerhans' Cell Density on Allergic Contact Dermatitis

ERICHARDT PROKSCHE AND JOCHEN BRASCH
Department of Dermatology, University of Kiel, Kiel, Germany

Previously, we have showed that artificial epidermal permeability barrier disruption leads to an increase in epidermal Langerhans' cell (LC) density within 24 h. We now asked if this is accompanied by an enhancement of allergic contact dermatitis. Barrier disruption was induced by acetone on the upper arms in 6 volunteers with known sensitization to nickel, fragrance mix, or p-phenylenediamine. Twenty-four hours after this treatment the relevant allergen was applied without occlusion or with Finn chambers. Twenty-four hours after application of the allergen, clinical grading and transepidermal water loss (TEWL) measurements were performed and biopsies were taken. Immunohistochemical stainings for LCs (anti-CD1a, Leu6) and for epidermal proliferation (Ki-S3) were performed.

Open applications of the allergens after acetone pretreatment resulted in strong allergic test reactions. TEWL, which showed a 70% recovery 24 h after acetone treatment, was increased again 4-fold by the allergic test reactions. LC density, which was increased by 80% 24 h after acetone-induced barrier disruption, was further enhanced 2.4-fold in total. Epidermal proliferation showed a 6-fold increase after open application of the allergens. Under patch test conditions after acetone pretreatment very strong bullish reactions were observed. We conclude that the increase in epidermal LC density induced by epidermal permeability barrier disruption is accompanied by an enhanced response in allergic contact dermatitis. Key words: transtunaneous water loss; epidermal proliferation.

(Accepted August 19, 1996.)
Acta Derm Venereol (Stockh) 1997; 77: 102–104.

E. Proksch, M.D., Ph.D., Department of Dermatology, University of Kiel, Schittenhelmstr. 7, DE-24105 Kiel, Germany.

We recently showed that acute disruption of the epidermal permeability barrier by open treatment with acetone, with sodium dodecylsulfate or by tape-stripping leads to a significant increase in epidermal Langerhans' cell (LC) density within 24 h (1). We now asked if such a barrier disruption and subsequent increase in LC density may lead to an enhanced response to contact allergens in sensitized patients.

MATERIALS AND METHODS

Disruption of the permeability barrier
Disruption of the permeability barrier was induced in 6 volunteers with known contact dermatitis to nickel sulfate (3 patients), fragrance mix (1 patient), or p-phenylenediamine (2 patients). For our studies we only used volunteers with a + to + + allergic reaction according to ICDRG-key, using the classical patch test chamber method. The study protocol was approved by the University of Kiel Human Research Review Committee. Either on the right or left upper arm, areas of approximately 12 x 5 cm were treated with acetone-soaked cotton balls by gently rolling for 20 min until a 10-20-fold increase in transepidermal water loss (TEWL) occurred (range 20-40 g/m2/h).

Measurements of TEWL were performed with an electronic water analyzer (Meeco, Inc. Warrington, PA, U.S.A.), as described previously (2). During the course of all experiments, the environmental humidity was 45±2%, and the room temperature was 22±1°C. TEWL measurements were performed immediately, 24, and 48 h after barrier disruption.

Patch tests
The acetone-treated site was divided into three areas. One area was left without further treatment, the second area was treated by open application of the allergen, and the third area was treated by the same amount of allergen + patch test chamber (Finn chamber). The allergens (nickel sulfate 5%, fragrance mix 8%, and p-phenylenediamine 1% in petrolatum; Heraeus-Chemie, Reinbek, Germany), were applied 24 h after barrier disruption (in 3 patients application was also performed immediately after barrier disruption.)

On the contralateral upper arm (without acetone pretreatment) one area was used as control and left untreated and the other area was treated by allergen + patch test chamber.

Twenty-four hours after application of the allergens the patch test chambers were removed. Clinical grading (ICDRG, International Contact Dermatitis Group (3)). TEWL measurements and punch biopsies were performed 24 h and 48 h after acetone treatment, or 24 h after application of the allergens.

Staining procedure and evaluation of slides
Immunohistochemical staining was performed on 6-μm cryostat sections. Monoclonal antibodies CD1a (CD1a, Becton Dickinson, Heidelberg, Germany) and Ki-S3 (proliferation-associated nuclear antigen P.R. Parwaresch, Kiel, Germany) were used for determination of epidermal LC density and epidermal proliferation, respectively. Reactivity was visualized with a standard biotin-avidin immunoperoxidase technique, using a commercially available kit (Vecta Stain, Carnon, Wiesbaden, Germany). The slides were evaluated using an Zeiss microscope, along with a 1-mm calibration grid, and the number of LCs (CD1a, Leu6) per mm length of basement membrane was counted (4, 5). The determination of LC densities per mm length of basement membrane gave reproducible results. The same results were obtained by counting LC density per mm length of stratum corneum (data not shown). We counted only those cells containing a nucleus and ignored the dendritic processes.

Ki-S3-positive cells were also counted per mm length of basement membrane. At each biopsy site, the mean number of CD1a and Ki-S3 positive cells was derived from the number of cells in each of three sections taken at 100-μm intervals (4).

Statistics
Statistical significance was determined using the paired Wilcoxon test.

RESULTS

TEWL in human skin after barrier disruption by acetone
Treatment with acetone resulted in an average 17-fold increase in TEWL. TEWL was 2.5 (range 2.1–3.1) g/m2/h before and 52.5 (range 39.8–65.6) g/m2/h after treatment. Barrier disruption was followed by a rapid recovery phase, leading to 70% recovery by 24 h (15.8 (range 12.1–18.3)) g/m2/h, p < 0.025. The treatment with open application of acetone was not of
relevant toxicity, because barrier disruption was followed by rapid recovery, and light microscopy examination did not show epidermal cell damage.

Clinical results (ICDRG-key) after allergen application ± acetone pretreatment

Treatment with acetone resulted in only moderate erythema which disappeared within the next 24 h. Open application of the allergens nickel, as well as fragrance mix or p-phenylenediamine 24 h after acetone pretreatment led to strong clinical reactions showing pronounced erythema and papulovesicles. Allergen application under patch test conditions (Finn chambers) after acetone pretreatment resulted in very strong reactions, showing erythema, vesicles and bullae. Without acetone pretreatment allergen application under patch test conditions was followed by a moderate test reaction, erythema and papules. After open application of an allergen without patch test chamber and without acetone pretreatment no clinical reaction was seen (data not shown). Open application of an allergen immediately after acetone-induced barrier disruption also resulted in a moderate + to ++ test reaction.

TEWL after allergen application

Forty-eight hours after acetone application TEWL decreased to 10.5 (range 7.4–13.2) g/m²/h (80% recovery) without allergen application. In contrast, open application of the allergens after acetone treatment resulted in a 3-fold increase in TEWL compared to the acetone-treated site (TEWL 52.0 (range 25.2–43.4) g/m²/h, p < 0.025) (12-fold increase compared to untreated). Allergen application with patch test chambers led to a very strong disruption of the epidermal permeability barrier (TEWL > 100.0 g/m²/h). Without acetone pretreatment, allergen application under patch test conditions also resulted in a significant increase in TEWL (TEWL 23.8 (range 19.7–33.8) g/m²/h (9.5 fold increase compared to untreated control). Open application of an allergen without acetone pretreatment or application of a patch test chamber without allergen did not lead to changes in TEWL (TEWL 2.4 ± 0.5 g/m²/h).

LC density after allergen application ± acetone pretreatment (Table 1)

Allergen application after acetone treatment led to an increase in LC density (2.4-fold increase compared to untreated control, p < 0.025). Allergen application with Finn chambers after acetone pretreatment also resulted in an increase in LC density, but to a lesser degree than after open applications (1.9-fold). Oclusive allergen application without acetone pretreatment was followed by a moderate increase (1.6-fold) in LC density compared to normal epidermis. Allergen application without acetone pretreatment and without occlusion did not influence LC density (data not shown).

Epidermal permeability barrier disruption by acetone led to a 68% increase in epidermal proliferation. A much stronger increase was obtained after allergen application following acetone pretreatment (6-fold compared to normal). Allergen application under patch test conditions after acetone pretreatment also resulted in a pronounced increase in epidermal proliferation, but to a lesser degree than without patch test chambers (4-fold). In contrast, allergen application with Finn chambers but without acetone pretreatment did not induce a significant change in epidermal proliferation.

DISCUSSION

Very recently we showed that barrier disruption by acetone treatment leads to an increase in epidermal LC density after 24 h (1). We now suggest that these LCs are functionally active because a strong patch test reaction was achieved by allergen application 24 h after acetone pretreatment, even without the use of patch test chambers. Following an increase due to barrier disruption, epidermal LC density was further enhanced by allergic test reactions. The highest numbers of LCs were obtained after open application of the allergens (2.4-fold in total). Oclusive allergen application led to a less pronounced increase (1.9-fold in total). We suggest that the strong inflammation induced by oclusive patch tests, leading to vesicles and bullae, depleted LCs from the epidermis. Occlusion after acetone-induced barrier disruption in humans enhances skin inflammation (E. Proksch: manuscript in preparation). This also explains why, without acetone pretreatment, the increase in LC density 24 h after allergen application by patch test chambers was only 63%. Different degrees of inflammation may explain why in previous studies an increase as well as a decrease in LC density in contact dermatitis has been described (6–9).

The increase in LC density 24 h after barrier disruption is of importance, because simultaneous barrier disruption by solvents and application of allergens does not enhance allergic test reactions (10). In contrast, when the application of irritants preceded antigen-challenge, a significant enhancement of patch test reactions was achieved (11, 12).

The mechanisms by which epidermal permeability barrier disruption increases LC density and enhances allergic reactions are still unknown. Activation of keratinocytes by solvents or irritants may lead to cytokine release. Previously, it was shown that epidermal permeability barrier disruption, as well as irritant contact dermatitis, induces the expression of the cytokine GM-CSF (13–15). GM-CSF regulates LC distribution in the lung (16).

| Table 1. LC density and Ki-S3 density after allergen application ± acetone pretreatment |
|-----------------|-----------------|-----------------|-----------------|
|                 | LC density      | Ki-S3 density   |                 |
|                 | (cells/mm²)     | (cells/mm²)     |                 |
| Untreated       | 2.08 (range 1.63–2.60) | 7.1 (range 5.8–9.0) |                 |
| Acetone         | 3.89 (range 2.80–4.93)* | 11.95 (range 9.3–16.9)* |                 |
| Acetone + allergen | 5.09 (range 3.01–6.10)* | 42.6 (range 32.2–68.6)* |                 |
| Acetone + allergen | 3.97 (range 3.01–6.10) | 29.2 (range 23.7–40.0)* |                 |
| patch test chamber |                  |                 |                 |
| Allergen + patch | 3.31 (range 2.68–4.87)* | 8.3 (range 6.0–10.0)* |                 |
| test chamber     | (without acetone) |                 |                 |

* Data = Mean and range.

p < 0.025.
It would also be of interest if barrier disruption and increase in LC density promote not only allergic test reactions but also sensitization to allergens. For ethical reasons we were unable to perform sensitization studies in humans.

Epidermal proliferation reached the highest values after open application of the allergens and was less pronounced under patch test chamber conditions. This confirms our previous findings that artificial barrier repair by latex occlusion after acetone treatment reduces the increase in epidermal proliferation (17). Occlusive conditions are also achieved by the use of patch test chambers.

Our results could be clinically relevant for the course of allergic contact dermatitis. It is well known that an irritant contact dermatitis (e.g. induced by repeated contact with solvents) may precede an allergic contact dermatitis. Our results indicate that this may not only be due to the disruption of the permeability barrier in irritant contact dermatitis, but also to the previously described increase in epidermal LC density in this disease (7). Therefore, to prevent relapses of allergic contact dermatitis in sensitized persons, it is very important to maintain their epidermal permeability barrier.

ACKNOWLEDGEMENTS

We thank C. Neumann for excellent technical assistance and Th. Henseler, M.D., Ph.D. for help in statistical evaluation. T. Evers capably performed typing of the manuscript. Supported in part by a grant from Deutsche Forschungsgemeinschaft (DFG) Bonn-Bad-Godesberg, Germany.

REFERENCES