

Regulation of the cutaneous allergic reaction by humidity

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Humidity is 1 of the environmental factors which regulate skin conditions. Effects of humidity on the cutaneous immune reaction were examined. Contact hypersensitivity to 2,4,6-trinitrochlorobenzene was elicited in C57BL/6 mice. The reaction was greater in mice housed under low humidity conditions (about 10%) for 2 days, at either the induction or elicitation phase, than in mice housed under rather high humidity conditions (80%). After housing under controlled humidity for 2 days, the number of I-A positive cells was 16% higher in the epidermis exposed to the dry condition. The increased population of FITC-positive cells were in regional lymph nodes after painting of FITC during housing under lower humidity. Our study demonstrated that the cutaneous immune reaction is regulated by environmental humidity and suggested 2 possible mechanisms, i.e., increase in Langerhans cells and increased penetration of allergen with low humidity.

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It has been observed that skin conditions change as environmental humidity changes. Klecz & Schwartz (1) reported that generalized pruritus may be caused by low humidity. Severity of skin disorders has been reported to increase in winter (2) and decreased humidity was suggested as causing the problem (3), whereas skin hydration appears to ameliorate these conditions (4). Regulation of the skin immune system may be, at least in part, 1 of the mechanisms of the phenomenon. However, effects of environmental humidity on the skin, especially the immune system, have not been studied.

We developed an isolator that can supply fresh air with regulated humidity. The humidity can be set to 10% or 80%. Using this apparatus, the effects of relative humidity were studied on epidermal barrier function (5), proliferation of epidermal cells (6, 7), and desquamation of stratum corneum (7).

The cutaneous immune reaction in mice housed in the isolator was analyzed in this report. Contact hypersensitivity (CH) was induced or elicited

under low or high humidity conditions. To examine the mechanism of the effects of environmental humidity, epidermal Langerhans cells (LC) were analyzed. LC are dendritic cells located at the lower epidermis. These cells present encountered antigens to T cells for induction and elicitation of immunity. The other factor examined was the penetration and/or transfer of the antigen. Migration of FITC molecules painted on the skin to regional lymph nodes was assayed.

Materials and Methods

Animals

Female C57BL/6 mice were purchased from Charles River Japan (Japan) and used for experiments at age 7 to 9 weeks. For regular housing, 5 mice were housed in a cage (225×338×140 mm) in a room with the temperature maintained at 21–25°C and relative humidity at 40–70%. For the experiments, 2 mice were housed in a cage (260×360×135 mm), kept in an isolator. The isolator supplies fresh air circulated 100× per h at controlled humidity, low (10%) or high (80%) (5). The temperature was 21–25°C and the NH₃ level was below 1 ppm. The study was approved by the Animal Research Committee of Shiseido Research

Abbreviations:

LC: Langerhans cells; CH: contact hypersensitivity; FITC: fluorescein isothiocyanate.

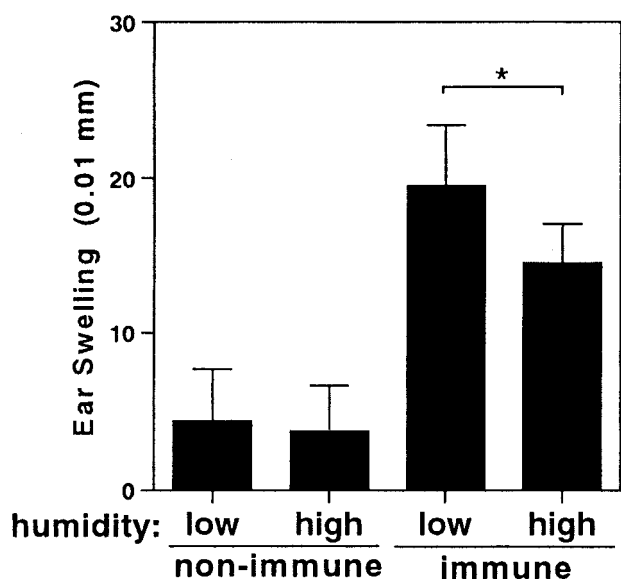


Fig. 1. Effects of humidity on elicitation of CH. CH was elicited during 2 days of housing under low or high humidity and 24-h swelling was measured. Representative data of 4 experiments. Bars, SD. * $p < 0.05$.

Center in accordance with the National Research Council Guide Lines.

Contact hypersensitivity reaction

Mice were immunized on day (D) 1 by application of 2,4,6-trinitrochlorobenzene, 50 μ l of 3% in acetone, on depilated dorsal skin. The contact hypersensitivity reaction was elicited on D6 by applying 5 μ l of 1% 2,4,6-trinitrochlorobenzene in acetone to each side of the right ear. The CH reaction was assessed by 24-h swelling measured with a micrometer (Mitsutoyo, Japan). To examine effects of humidity on induction of immunity, mice were kept in the humidity-controlled isolator for 48 h, D0 through D2. For examination of effects on elicitation of CH, mice were kept in the isolator for 48 hours, D5 through D7. Every experimental group consisted of 8 or 10 mice.

Staining of Langerhans cells

Epidermal LC were stained as described elsewhere. Briefly, the epidermis was separated and removed from the dermis by incubation in 3.8% ammonium thiocyanate at 37°C for 20 min. The epidermal sheets were fixed with acetone and incubated in the culture supernatants of mouse hybridoma, M5/114.15.2 (American Tissue Type Collection, Rockville, USA), secreting anti-I-A IgG, and then in anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) (Sigma, USA). Cells positive for I-A were analyzed by a confocal laser scanning

microscope (MRC600, BioRad, USA). Samples were collected from 6 mice per group. 3 fields of each sample were analyzed in blind fashion.

Analysis of FITC-bearing LC in regional lymph nodes

10 μ l of 3% FITC in acetone were applied on each side of both ears after housing under low or high humidity for 24 h, followed by subsequent 24-h housing under the same conditions. Cells isolated from 20 cervical lymph nodes collected from 5 mice were analyzed by a flowcytometer (Beckman Coulter, Japan). Experiments were repeated 2 $\times$.

Statistical analysis

Differences between groups were analyzed by the Student *t*-test.

Results

Regulation of contact hypersensitivity by humidity

The effects of humidity on the cutaneous immune reaction were examined. Contact hypersensitivity was elicited during housing under low or high humidity for 48 h. The reaction was greater in mice housed under lower humidity (Fig. 1). Non-specific reaction in nonimmunized mice was consistently about 0.04 mm under regular conditions and was

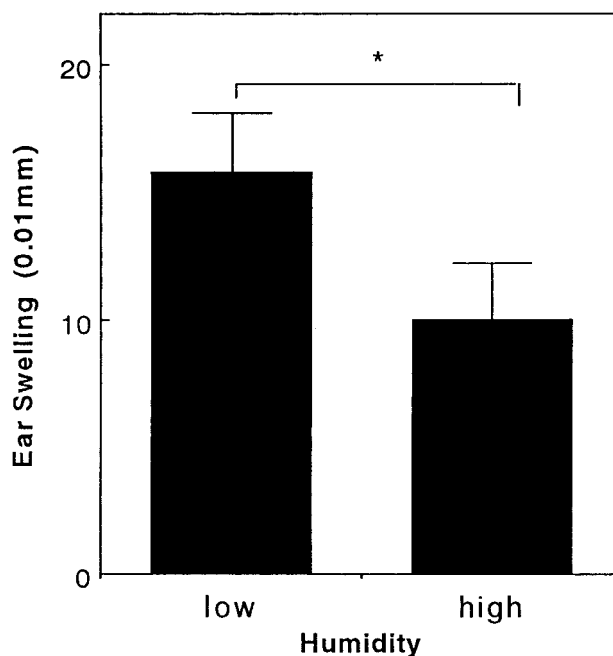


Fig. 2. Effects of humidity on induction of CH. Mice were sensitized during 2 days of housing under low or high humidity and 24-h swelling was measured. Representative data of 3 experiments. Bars, SD. * $p < 0.05$.

Table 1. Effects of humidity on LC phenotype

Humidity	No. of LC (cells/mm ²)	Intensity of I-A	Area per cell (μm^2)	No. of dendrites
low	95.0 (9.1)	35.6 (3.9)	156.1 (12.5)	2.75 (0.24)
high	81.6 (7.0) *	38.5 (10.7)	171.8 (7.7) *	2.93 (0.16)

Epidermal sheets were removed after housing under low- or high-humidity conditions, stained for I-A, and analyzed by confocal laser scanning microscope. SD in parentheses.

* $p < 0.05$.

not affected by humidity. A difference in the reaction was also observed in mice sensitized under controlled humidity. When mice were sensitized at the middle of a 48-h period of housing at low humidity, the contact hypersensitivity reaction elicited under regular conditions was higher (Fig. 2). These results demonstrate that environmental humidity affects the cutaneous immune reaction.

Regulation of epidermal LC by humidity

To examine the effects of humidity on epidermal LC, we analyzed I-A-positive cells in the epidermis in mouse ears after housing under controlled humidity for 48 h. There were more I-A-positive cells in the epidermis of mice exposed to lower humidity (Table 1). These results suggest that environmental humidity affects epidermal LC, resulting in regulation of the immune reaction. Unexpectedly, other

parameters were similar or lower in mice housed under lower humidity.

Migration of allergen-bearing cells into regional lymph nodes

Antigen penetration of skin and transfer into regional lymph nodes was assessed by analyzing FITC-bearing cells in regional lymph nodes after painting with FITC. As shown in Fig. 3, about $2\times$ as many cells were FITC-positive in mice exposed to lower humidity (1.9% in lower humidity, 1.0% in higher humidity). These results suggest that more allergen is transferred to the regional lymph nodes under lower humidity conditions.

Discussion

The cutaneous immune reaction was found to be affected by environmental humidity. Both the induction and elicitation of immunity for CH was higher in mice housed at lower humidity, suggesting that humidity modified the skin immune system. Other climatic parameters, including temperature, airflow, and NH_3 were identical in the low- and high-humidity cages. Animals were not directly exposed to the air stream. There was little difference in body weight between mice housed under low- and high-humidity conditions. These data strongly suggest that humidity was the factor that regulated the immune reaction. The data on non-specific reaction in non-immunized mice also support the idea. However, various irritants must be examined to conclude the effect of humidity on irritation.

During the elicitation phase of CH, antigens are presented by LC to circulating T cells at the site of the lesion. The immune system of the whole body might be affected by environmental humidity along with LC. Ashida et al. (8) found that IL-12 and IgG increased under lower humidity. Interestingly, they also found that some skin parameters, such as transepidermal water loss, were decreased.

The induction of immunity was also affected by environmental humidity. In this phase, LC capture antigens in the epidermis and migrate to the local lymph nodes, where antigens are presented to

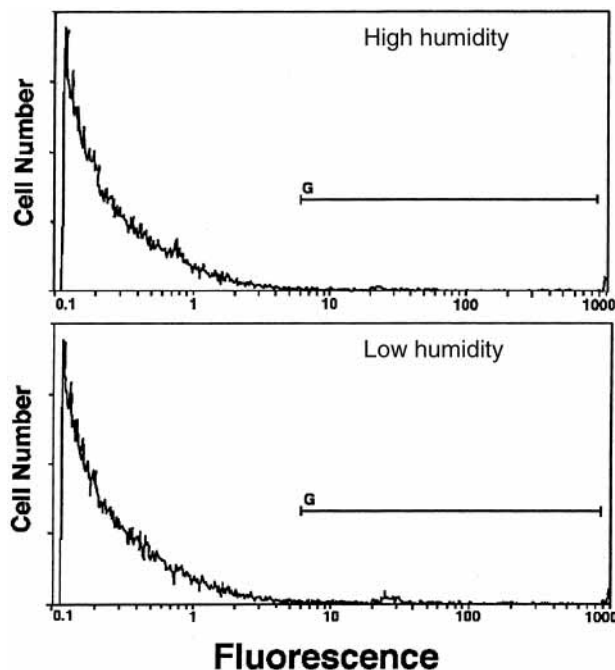


Fig. 3. Effects of humidity on migration of hapten-bearing cells. Cells isolated from regional lymph nodes after FITC application were analyzed by flowcytometry. Representative data of 2 experiments.

naïve T cells. Therefore, the rôle of LC must be considered. We found that epidermal LC were increased under lower humidity. This might be 1 mechanism of the effect of low humidity. The dry condition induces impairment of desquamation (7). Proksch & Brasch (9) disrupted the epidermal permeability barrier by acetone treatment and found an increase in LC density. Changes in the upper layers of the epidermis may affect LC.

FITC-bearing cells in regional lymph nodes were increased after housing under dry conditions. LC migrate from the epidermis to regional lymph nodes, when they are stimulated by antigens, and present the antigen to naïve T cells. It is also possible that penetrated free FITC molecules migrate to regional lymph nodes and are captured by certain antigen-presenting cells. Penetration of antigens through the cornified layer of the epidermis may be upregulated. This might be another mechanism of the effect of low humidity. Both increase in LC and in penetration of foreign antigens may have resulted in activating the induction of immunity.

Low humidity is considered to be 1 of the factors causing skin problems (2). Our results provided evidence of the effects of environmental humidity on the cutaneous immune reaction. These results could help to clarify the mechanisms of maintaining healthy skin, as well as the development of various skin disorders.

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