Hyperthermia Depletes Epidermal Langerhans Cells and Modulates Contact Hypersensitivity Reaction in Mice

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This study was designed to investigate the effect of local hyperthermia on contact hypersensitivity (CHS) and elucidate its mechanism through assessment of number of epidermal LCs and transfer of spleen cells. Depilated dorsal skin of mouse was immersed into controlled water bath at 52 °C for 30 seconds in vivo. The number of epidermal LCs was counted by adenosine triphosphate staining, and CHS to 2,4-dinitro-1-fluorobenzene was assessed by ear swelling and transfer of spleen cells.

The number of LCs was significantly reduced 1 to 3 days after the hyperthermia treatment and recovered to normal 5 days after the treatment. CHS was significantly suppressed in mice sensitized 5,7, or 10 days after hyperthermia treatment, but the suppression was meager in mice sensitized 1 or 3 days after the treatment. There is a discord between the number of LCs and degree of CHS.

When mice received spleen cells from hyporesponsive donors, CHS was remarkably suppressed in the recipient mice compared with positive control. These findings suggest that treatment of local hyperthermia suppress CHS in mice, which may be associated with the induction of suppressor cells. The nature of the discord between the number of LCs and degree of CHS in this investigation remains to be cleared by further studies. (Ann Dermatol 2:2(2) 71-76, 1990)

Key Words: Contact hypersensitivity, Hyperthermia, Langerhans cells.

The concept that epidermal Langerhans cells (LCs) play a central role in the induction of contact hypersensitivity (CHS) was first suggested by their special affinity for several contact allergens and their apposition to lymphocytes at sites of elicitation reactions. The number of epidermal LCs is depleted or reduced by various stimuli (e.g., ultraviolet irradiation, roentgen rays, corticosteroids and chemical carcinogen). The immunological changes following burns, have received considerable attentions. Interestingly, Morhenn et al. demonstrated that the viability of LCs was much more sensitive to hyperthermia treatment than that of other epidermal cells in vitro study and hyperthermia pretreatment of dispersed skin cells inhibited mixed epidermal cell-lymphocyte reaction.

Ultraviolet B (UVB) has been regularly shown to decrease the density of LCs. There have been numerous studies of the effects of UVB on CHS model. UVB irradiated mice display a marked depression of CHS reactions to topically applied contact sensitizing agents. These changes undertake one of two forms depending on the dose of UVB before sensitization, local and systemic suppression of CHS.

This study was designed to investigate the suppressive effect of local hyperthermia on CHS and elucidate its mechanism through assessment of number of epidermal LCs and transfer of spleen cells.

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MATERIALS AND METHODS

1. Experimental animals and hyperthermia treatment
A total of 67 female C3H/HeN mice, 8-10 weeks old, were used. All mice were obtained from Genetic Engineering Center, Korea Advanced Institute of Science and Technology and were rest ed for at least 2 weeks before use. Twelve mice were allocated for staining of LCs. Each experimental group, positive control group and negative control group consisted of 5 animals.

Depilated dorsal skin was immersed in a temperature controlled water bath, 52°C for 30 seconds.

2. Staining of LCs.
For the identification of LCs, Mackenzie and Squier method with minor modification was used. Briefly, the epidermis was separated from the underlying dermis by incubation in buffered EDTA for 2h at 37°C. The separated epidermis was washed in cold, 0.2M Tris buffer (pH7.3) for 30 min and then immersed in a cacodylate-formaldehyde solution for 20 min 4°C. After rinsing in Tris buffer, the epidermis was incubated at 37°C for 40 min in a substrate consisting of 10 mg ATP (Sigma Chemical Co., St. Louis, Missouri), 5 ml 5% MgSO4, 3ml 2% PbNO3 in 42 ml Tris buffer. After through wash in Tris buffer, the sheets were treated with a 5% ammonium sulfide solution for 1 min. After final wash in distilled water, the sheets were mounted on microscope slides in glycerol. Count of LCs was done in mice received hyperthermia treatment 1.3,5,7 or 10 days previously and untreated mice. The number of AT-Pase positive epidermal cells was determined by randomly counting 10 fields at ×400 with an ocular grid of known area, and was expressed as mean number of cells per square millimeter.

3. Assessment of CHS
For sensitization, a solution of 50 ul of 0.5% 2,4-dinitro-1-fluoro-benzene (DNFB, Sigma) in acetone was applied epicutaneously on the shaved back skin which received hyperthermia treatment. Five days after the sensitization, the animals were challenged on each surface of both ears with paint ing of 5 ul of 0.25% solution of DNFB in acetone. Sensitization was done 1, 3, 5, 7 or 10 days after hyperthermia treatment. The positive control which was sensitized and challenged, and the negative control which was challenged but not sensitized was not received hyperthermia treatment.

The extent of ear swelling was used to measure CHS, and is expressed as the difference in thickness between before and 24h after ear challenge measured with engineer’s micrometer (Mitutoyo, Tokyo, Japan). The percentage of suppression of CHS responses in hyperthermia-treated animals was calculated by the following formula:

\[ \% \text{ suppression} = 1 - \frac{\text{experimental-negative}}{\text{positive-negative}} \times 100 \]

4. Cell transfer
Spleen cells obtained from hyposensitive mice and positive control mice 6 days after sensitization were injected into tail vein of normal syngeneic mice. Single cell suspensions were prepared by teasing the spleen with needles in RPMI 1640 media (Sigma) containing penicillin (100U/ml) and streptomycin (100 ug/ml). The viable cell value, as assessed by trypan blue dye exclusion, was always greater than 90%. Each mouse received 1x10⁸ cells in 0.5ml of RPMI 1640 media intravenously. Two hours after cell transfer, the recipient mice were sensitized on the back with 0.5% DNFB in acetone.

5. Statistical analysis
Differences among measns for experimental panels and positive controls were calculated with Student’s t-test.

RESULTS

1. Effect of local hyperthermia on the density of epidermal LCs
The skin showed neither crust formation grossly nor pyknosis of cells microscopically by this hyperthermia. As shown in table 1, the density of LCs decreased to 56.2% compared with control value one day after hyperthermia treatment (P<0.001). Three days after hyperthermia treatment, the density of LCs recovered to 77.0% of control value (P<0.001) and it showed nearly nor-
normal density 5, 7 or 10 days after hyperthermia treatment. This finding indicated that ATPase-positive LCs recovered within a relatively short period following exposure to hyperthermia treatment.

2. Effect of local hyperthermia on CHS to DNFB

As shown in table 2, local hyperthermia treatment significantly suppressed the CHS reaction to DNFB when the mice were sensitized on the treated skin 5, 7 or 10 days after hyperthermia treatment compared with positive control ($P < 0.001$, $P < 0.001$, $P < 0.01$). When the mice were sensitized 1 or 3 days after hyperthermia treatment, nearly normal CHS reaction was observed.

3. Correlation between the degree of CHS and the number of LCs in epidermis

When mice were sensitized 1 or 3 days after hyperthermia treatment, CHS was meagerly suppressed while the number of ATPase-positive LCs was remarkably reduced. When mice were sensitized, 5, 7 or 10 days after hyperthermia treatment, CHS was remarkably suppressed but the number of ATPase-positive LCs was nearly recovered to normal value (Fig. 1).

4. Transfer of CHS by Spleen Cells

Cell transfer was performed to demonstrate whether CHS suppressed by hyperthermia was transferable (Table 3).

CHS reaction was remarkably suppressed when spleen cells were transferred from hyporesponsive donors sensitized 5 or 7 days after hyperthermia treatment ($P < 0.001$).

### Table 1. Effect of local hyperthermia on the density of ATPase-positive Langerhans cells

<table>
<thead>
<tr>
<th>Days after hyperthermia</th>
<th>ATPase-Positive LCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>density $/\text{mm}^2$</td>
</tr>
<tr>
<td>1</td>
<td>456±58.5§</td>
</tr>
<tr>
<td>3</td>
<td>625±35.2</td>
</tr>
<tr>
<td>5</td>
<td>767±44.0</td>
</tr>
<tr>
<td>7</td>
<td>782±57.1</td>
</tr>
<tr>
<td>10</td>
<td>799±42.7</td>
</tr>
<tr>
<td>Positive control#</td>
<td>812±81.9</td>
</tr>
</tbody>
</table>

LCs: Langerhans cells
§ Mean value ± S.D.
* $P < 0.001$

# Mice which were not treated with hyperthermia.

### Table 2. Effect of local hyperthermia on contact hypersensitivity

<table>
<thead>
<tr>
<th>Days after Hyperthermia</th>
<th>DNFB sensitization</th>
<th>Ear swelling response ($\times 10^{-2}$ mm)</th>
<th>Percent suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>9.6±1.8§</td>
<td>18.9</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>9.8±3.0</td>
<td>16.8</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>6.3±1.3</td>
<td>53.7**</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>6.5±1.2</td>
<td>51.6**</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>8.3±1.2</td>
<td>32.6</td>
</tr>
<tr>
<td>Positive control#</td>
<td>+</td>
<td>11.4±2.6</td>
<td>—</td>
</tr>
<tr>
<td>Negative control#</td>
<td>—</td>
<td>1.9±0.7</td>
<td>—</td>
</tr>
</tbody>
</table>

§ Mean value ± S.D.
* $P < 0.01$
** $P < 0.001$

# Mice which were not treated with hyperthermia.
Fig. 1. Correlation between the degree of contact hypersensitivity and the number of epidermal Langerhans cells
LCs: Langerhans cells
NS: Statistically nonsignificant
# Mice which were not treated with hyperthermia.

Fig. 2. Epidermal Langerhans cells (LCs) from mouse skin without hyperthermia treatment. LCs are shown as dendritic dark brown cells (ATPase staining, ×400).

Fig. 3. Epidermal LCs 1 day after hyperthermia treatment (30 sec at 52°C), showing marked reduction in number and loss of dendrites (ATPase staining, ×400).

Fig. 4. Epidermal LCs 3 days after hyperthermia treatment, showing moderate recovery in number (ATPase staining, ×400).

Fig. 5, 6, 7. Epidermal LCs 5, 7 and 10 days after hyperthermia treatment, showing nearly complete recovery in number (ATPase staining, ×400).
Table 3. Transfer of contact hypersensitivity by spleen cells

<table>
<thead>
<tr>
<th>Days after hyperthermia in donors</th>
<th>DNFB sensitization of cell donors</th>
<th>DNFB sensitization of recipients</th>
<th>Ear swelling response (×10⁻³:mm)</th>
<th>Percent suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>6.9±1.9$§</td>
<td>58.7$</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>7.1±2.0</td>
<td>60.4$</td>
</tr>
<tr>
<td>Positive control#</td>
<td>+</td>
<td>+</td>
<td>13.2±1.9</td>
<td></td>
</tr>
<tr>
<td>Negative control##</td>
<td>-</td>
<td>-</td>
<td>2.8±1.2</td>
<td></td>
</tr>
</tbody>
</table>

DNFB: 2,4-dinitro-1-fluorobenzene  
# Donor mice were not treated with hyperthermia.  
§ Mean value ± S.D.  
## Mice were only challenged.

DISCUSSION

LCs play a crucial role in the afferent limb of the immune response. They act as the principal antigen-presenting cells for delayed type hypersensitivity reactions in vivo including the induction of CHS. They are also required for in vitro epidermal cell-induced, T cell-mediated responses to foreign protein and alloantigen. Morhenn et al. demonstrated that LCs were much more sensitive to hyperthermia treatment in vitro study and hyperthermia treatment inhibited mixed epidermal cell-lymphocyte reaction. In our in vivo study, a temporary decrease in the number of epidermal LCs after hyperthermia treatment was observed. The density of ATPase-positive LCs was markedly decreased 1 day after hyperthermia treatment and returned to nearly normal level in 5 days. UVB exposure has been well known to decrease the density of LCs. ATPase-positive LCs were depleted 2 days after a single exposure to low dose of UVB and recovered 2-3 weeks after this irradiation. Immediately after exposure to low dose of UVB daily for 4 consecutive days, ATPase-positive LCs were depleted, and these cells recovered 2 weeks after this irradiation. Jun et al. demonstrated that the density of ATPase-positive cells returned to normal value 5 days after exposure to low or high dose of UVB.

In the present experiment, we demonstrated that hyperthermia treatment suppressed CHS to DNFB in mice when sensitized 5 days to 10 days after the treatment. In a report by Roszkowski et al., mice were exposed to whole-body microwave hyperthermia treatment 2 h daily for 4, 7, 10 or 14 days and the each group was sensitized with oxazolone. From group treated with this hyperthermia for 7 days CHS response level was significantly suppressed and CHS response returned to normal when sensitization was performed 8 days after this hyperthermia treatment for 7 days. In our study, a single and local exposure to hyperthermia exerted a suppressive effect on CHS and this effect is comparable to CHS response to DNFB suppressed immediately after exposure to low or high dose of UVB which persisted for at least 7 days after exposure.

Toews et al. indicated a direct correlation between the number of ATPase-positive LCs in epidermis and the ability to induce CHS reactions. But Lynch et al. could not demonstrate this correlation. In our study, a meager suppression of CHS was observed in mice sensitized on LGs-depleted skin 1 or 3 days after hyperthermia treatment. This result was not observed in previous reports about effect of UVB on CHS, but was relatively consistent with that by tape stripping in experiment of Baker et al. The epidermal LCs were depleted by tape stripping but this treatment did not alter the degree of CHS. In our study, CHS reaction was suppressed in mice sensitized on LCs-repleted skin since 5 days after hyperthermia treatment. This result was consistent with previous reports about local effect of UVB on CHS. However, this disproportion may represents the time lag between the LCs depletion and the generation of the specific suppressor cells after hyperthermia.
Suppressive effect of UVB on CHS is considered to be mediated in part by antigen-specific suppressor T cells. The present study demonstrated that suppressive effect of local hyperthermia on CHS could be transferred into normal mice by spleen cells generated by pre-exposure of the sensitizing site to hyperthermia treatment.

Taken together, local hyperthermia treatment suppresses CHS in mice and this suppressive effect seems to be in part associated with the induction of suppressor cells. However, antigen specificity and phenotype of the suppressor cells, and the disproportion between the degree of CHS and the number of LCs after hyperthermia treatment remain to be addressed.

REFERENCES