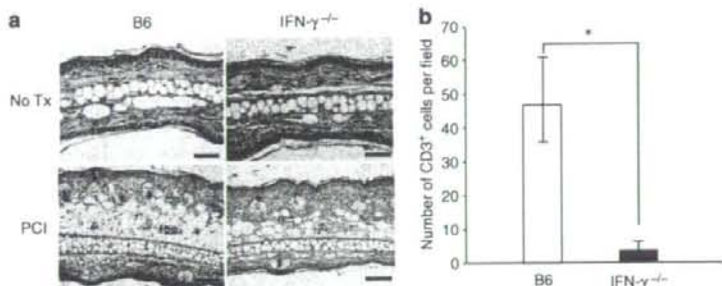

Figure 2. Histology and numbers of CD3$^+$ T cells in PCI-challenged skin of B6 and IFN-γ$^{-/-}$ mice. (**a**) B6 and IFN-γ$^{-/-}$ mice were sensitized with 5% PCI and challenged with 0.5% PCI. The challenged ears, along with no-treated ones (no Tx), were stained with hematoxylin and eosin. (**b**) Cryostat sections of the challenged ears were immunohistochemically stained with anti-CD3 mAb, and CD3$^+$ cells were enumerated in original magnification × 500 field. Data are expressed as the mean ± SD of five mice. *P = 0.00018. Bar = 80 μm.

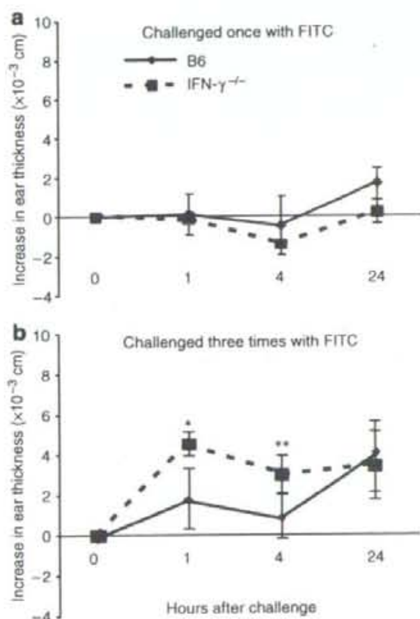

Figure 3. Time course of ear swelling responses of B6 and IFN-γ$^{-/-}$ mice receiving single-time or three time-elicitation with FITC. B6 and IFN-γ$^{-/-}$ mice were sensitized with 1% FITC on the abdomen and challenged with 0.5% FITC once or thrice (3- to 4-day intervals) on the ears. One, four, and 24 hours after the final challenge, the ear swelling responses were measured. Data are expressed as the mean ± SD of five mice. *P = 0.0048, **P = 0.0060.

24 hours after challenge in either strain of mice (Figure 3a). When challenged three times, significantly higher swelling responses were found at 1 and 4 hours in IFN-γ$^{-/-}$ mice than in B6 mice (Figure 3b). There was no difference between the two strains at 24 hours. The six-time challenge induced similar but slightly higher levels of these reactions (data not shown). The results suggested that IFN-γ$^{-/-}$ mice are prone to develop immediate and late-phase cutaneous reactions instead of DTH, suggesting a Th2-skewed immunological state.

Augmentation of IL-4 expression by repeated FITC challenge in immune lymph nodes of IFN-γ$^{-/-}$ mice

To confirm the preponderant expression of Th2 cytokines in IFN-γ$^{-/-}$ mice, B6 and IFN-γ$^{-/-}$ mice were sensitized with PCI or FITC, and challenged once with PCI or six times with FITC, respectively. Cervical lymph nodes were taken from these mice 24 hours after the last challenge, and IFN-γ and IL-4 expressions were examined by real-time PCR. B6 mice had IFN-γ expression at a higher level by single challenge with PCI than by repeated challenge with FITC, whereas IFN-γ$^{-/-}$ mice naturally exhibited no IFN-γ expression (Figure 4). As for IL-4, the repeated FITC challenge enhanced its expression in both strains of mice as compared to PCI challenge, but the level of enhancement by FITC was greatly higher in IFN-γ$^{-/-}$ mice than in B6 mice. These data indicated preferential activation of Th2 cells in IFN-γ$^{-/-}$ mice after repeated FITC challenge.

Infiltration of Th2 cells and presence of IgE and IL-4 at repeatedly FITC-challenged skin of IFN-γ$^{-/-}$ mice

B6 and IFN-γ$^{-/-}$ mice were sensitized and challenged six times with FITC, and their ears were histologically examined. Both types of mice exhibited an inflammatory infiltrate mainly in the dermis (Figure 5a). Some lymphocytes infiltrated into the epidermis with spongiosis. Immunohistochemically, IgE and IL-4 were present in IFN-γ$^{-/-}$ mice presumably in association with mast cells and T cells, respectively (Figure 5b).

Epidermal cell suspensions, including epidermotropic lymphocytes as well as ECs, were prepared from the challenged skin and subjected to real-time PCR analysis for two chemokine receptors. CXCR3 is expressed mostly on Th1 cells, and CCR4 is preferentially on Th2 cells (Sebastiani *et al.*, 2002). Whereas CXCR3 was markedly expressed by FITC challenge in B6 but not in IFN-γ$^{-/-}$ mice, CCR4 expression was dramatically increased by repeated FITC painting in IFN-γ$^{-/-}$ but not in B6 mice (Figure 6). Taken together with the presence of IL-4 in IFN-γ$^{-/-}$ mice challenged with FITC, this finding provides a possibility that IFN-γ deficiency allows Th2 cells to infiltrate into the skin, leading to the occurrence of immediate and late-phase reactions.

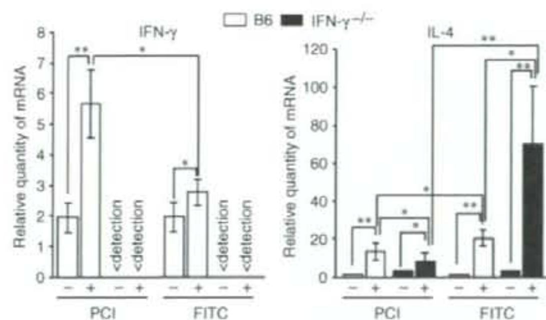

Figure 4. Real-time PCR analysis of mRNA expression for cytokines in cervical lymph node cells. B6 and IFN-$\gamma^{-/-}$ mice were sensitized with 5% PCl and challenged once with 0.5% PCl, or sensitized with 1% FITC and challenged six times with 0.5% FITC (twice a week for 3 weeks). Single-cell suspensions of immune cervical lymph node cells were prepared and subjected to real-time PCR analysis for IFN-γ and IL-4. Data are expressed as the mean ± SD of four mice. $^*P<0.05$, $^{**}P<0.005$.

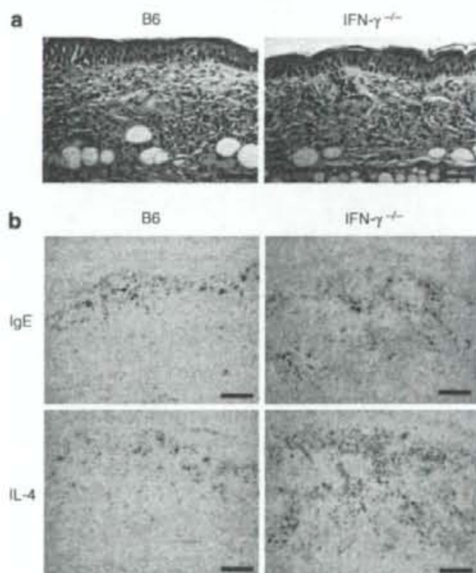

Figure 5. Histology and immunohistochemical stainings for IgE and IL-4 in B6 and IFN-$\gamma^{-/-}$ mice receiving six times repeated elicitation with FITC. (**a**) B6 and IFN-$\gamma^{-/-}$ mice were sensitized with 1% FITC and challenged with 0.5% FITC repeatedly six times (twice a week). The challenged ears were stained with hematoxylin and eosin. (**b**) Cryostat sections of the challenged ears were immunohistochemically stained with anti-IgE or anti-IL-4 mAb. Bar = 50 μm.

Reduced CHS response to PCl in IFN-$\gamma^{-/-}$ mice receiving adoptive transfer

Draining lymph node cells (5×10^6 cells) from wild-type B6 mice that were sensitized with PCl 5 days before were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and transferred to naive B6 or IFN-$\gamma^{-/-}$ mice.

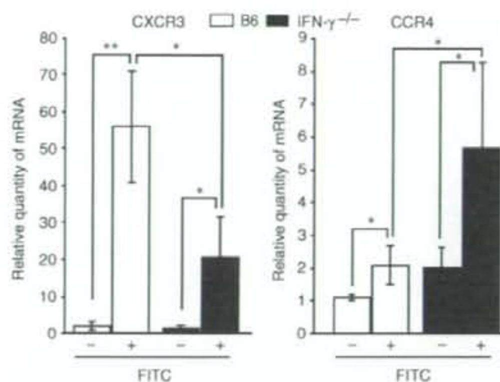

Figure 6. Real-time PCR analysis of mRNA expression for chemokine receptors in skin-infiltrating cells. B6 and IFN-$\gamma^{-/-}$ mice were sensitized with 5% PCl and challenged once with 0.5% PCl, or sensitized with 1% FITC and challenged six times with 0.5% FITC (twice a week for 3 weeks). Single-cell suspensions of epidermal and dermal cells were prepared and subjected to real-time PCR analysis for CXCR3 and CCR4. Data are expressed as the mean ± SD of four mice. $^*P<0.05$, $^{**}P<0.005$.

Immediately after transfer, mice were challenged with PCl on their ears. Although B6 mice transferred with sensitized lymph node cells exhibited a significantly high ear swelling response, IFN-$\gamma^{-/-}$ mice failed to respond to the challenge (Figure 7a). This result clearly indicated impairment of the efferent phase of CHS in IFN-$\gamma^{-/-}$ mice. When the ears of mice receiving CFSE-labeled lymph node cells were histologically examined 24 hours after challenge, the number of CFSE-positive cells in the ears of IFN-$\gamma^{-/-}$ mice was significantly lower than that of B6 mice (Figure 7b).

To further confirm this paucity of infiltration, draining lymph node cells (5×10^6 cells) from green fluorescent protein (GFP) mice sensitized with DNFB 5 days before were transferred to naive B6 or IFN-$\gamma^{-/-}$ mice. The recipients were immediately challenged with DNFB. B6 but not IFN-$\gamma^{-/-}$ mice had a high ear swelling response (Figure 7c). Again, histological examination at 24 hours after challenge showed less infiltration of GFP-positive lymphocytes in IFN-$\gamma^{-/-}$ mice (Figure 7d).

To test the participation of IFN-γ in the afferent limb, immune lymph node cells from B6 or IFN-$\gamma^{-/-}$ mice sensitized with PCl were transferred to naive B6 mice, and the recipient mice were challenged with PCl. The CHS response of recipients receiving sensitized lymph node cells of IFN-$\gamma^{-/-}$ mice was lower than that of mice receiving B6 lymph node cells (Figure 7e). This suggests that IFN-γ is involved in both induction and elicitation of CHS.

Reduction of Th1 chemokine production by keratinocytes in IFN-$\gamma^{-/-}$ mice and enhancement of Th2 chemokine production by LCs in IFN-$\gamma^{-/-}$ mice

Epidermal cell suspensions were obtained from the ears of B6 and IFN-$\gamma^{-/-}$ mice that were challenged once with PCl or six times with FITC. Crude ECs were analyzed for EC-derived chemokines. Both challenges with PCl and FITC elevated the

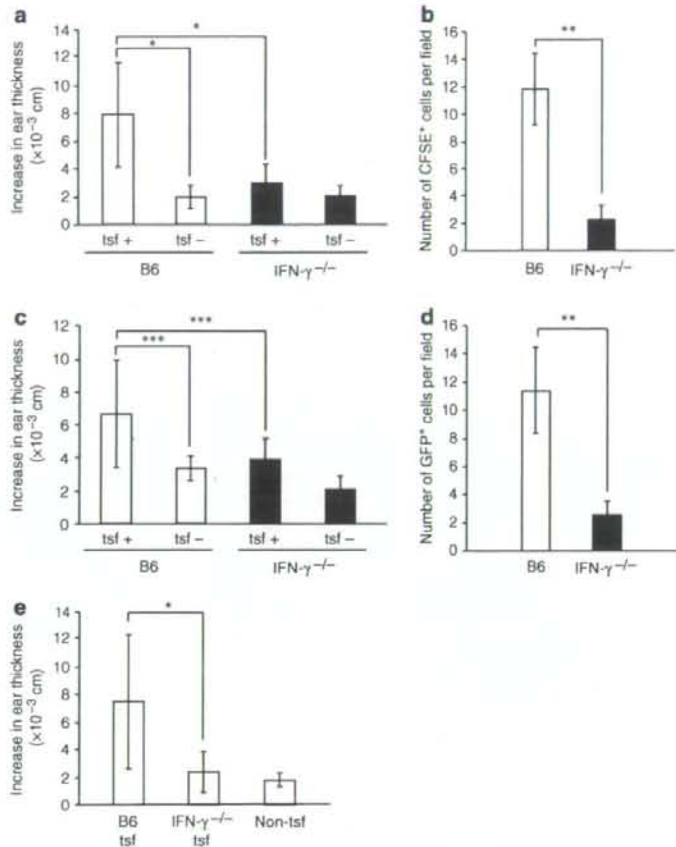

Figure 7. Adoptive transfer of CHS using immune lymph node cells labeled with CFSE and those from GFP mice. (**a**) Axillary, bradial, and inguinal lymph node cells (5×10^6 cells) of B6 mice that were sensitized 5% PCI on the shaved abdomen 5 days before were labeled with CFSE and transferred to naive B6 or IFN-$\gamma^{-/-}$ mice. Immediately after transfer (tsf), mice were challenged with 10 µl of 0.5% PCI to both sides of ears (tsf +). Non-transferred B6 and IFN-$\gamma^{-/-}$ mice were described as tsf−. The increment of ear swelling was measured at 24 hours. (**b**) Ears of B6 and IFN-$\gamma^{-/-}$ mice receiving transfer of CFSE-labeled immune lymph node cells from PCI-sensitized mice were taken 24 hours after transfer and subjected to the histological study. The number of CFSE-positive cells in the dermis was counted in six different original magnification ×200 power fields and represented by the mean. (**c**) Immune lymph node cells were prepared from GFP mice sensitized with 0.5% DNFB and transferred to naive B6 or IFN-$\gamma^{-/-}$ mice. Mice were challenged with 10 µl of 0.3% DNFB to both sides of ears (tsf +). Non-transferred mice were represented as tsf−. The increment of ear swelling was measured at 24 hours. (**d**) Ears of B6 and IFN-$\gamma^{-/-}$ mice receiving transfer of immune lymph node cells (5×10^6 cells) from DNFB-sensitized GFP mice were taken 24 hours after transfer and subjected to the histological study. The number of GFP-positive cells in the dermis was counted in six different original magnification ×200 power fields and represented by the mean. (**e**) Lymph node cells were prepared from B6 or IFN-$\gamma^{-/-}$ mice sensitized with 5% PCI and transferred to naive B6 mice. The recipient mice were challenged with 10 µl of 0.5% PCI (B6, IFN-$\gamma^{-/-}$). The non-transferred mice represent the group of mice challenged alone. The ear thickness change was measured at 24 hours. Data are expressed as the mean ± SD of four mice. $^{*}P<0.001$, $^{**}P<1.0 \times 10^{-6}$, and $^{***}P<0.05$.

expression of Th1 chemokine CXCL9 and CXCL10 in B6 mice, whereas such an augmentation was not found in IFN-$\gamma^{-/-}$ mice (Figure 8, left). As for Th2 chemokines, PCI challenge induced the expression of CCL17 and CCL22 in both B6 and IFN-$\gamma^{-/-}$ mice. Repeated FITC challenge augmented the expression of Th2 chemokines CCL17 and CCL22 in IFN-$\gamma^{-/-}$, but FITC augmentation for CCL17 and CCL22 was minimal in B6 mice. It was reported that CCL27/CTACK-CCR10 signaling is an important regulator for skin immune inflammation (Homey *et al.*, 2002). Therefore, CCL27 expression was also monitored, but there was no substantial difference between challenged and non-treated ears, or between B6 and IFN-$\gamma^{-/-}$ mice. Only marginal increment was observed in IFN-$\gamma^{-/-}$ mice that were challenged six times with FITC compared to non-treated IFN-$\gamma^{-/-}$ mice (relative quantitative mRNA: 3.1 vs 1). Accordingly, no enhanced expression of CCR10, a ligand for CCL27, was found in PCI- or FITC-challenged skin of B6 or IFN-$\gamma^{-/-}$ mice.

When CD11c^{+} cells, namely LCs in the epidermis, were depleted from crude EC suspensions, and again subjected to real-time PCR analysis, signals of Th2 chemokines

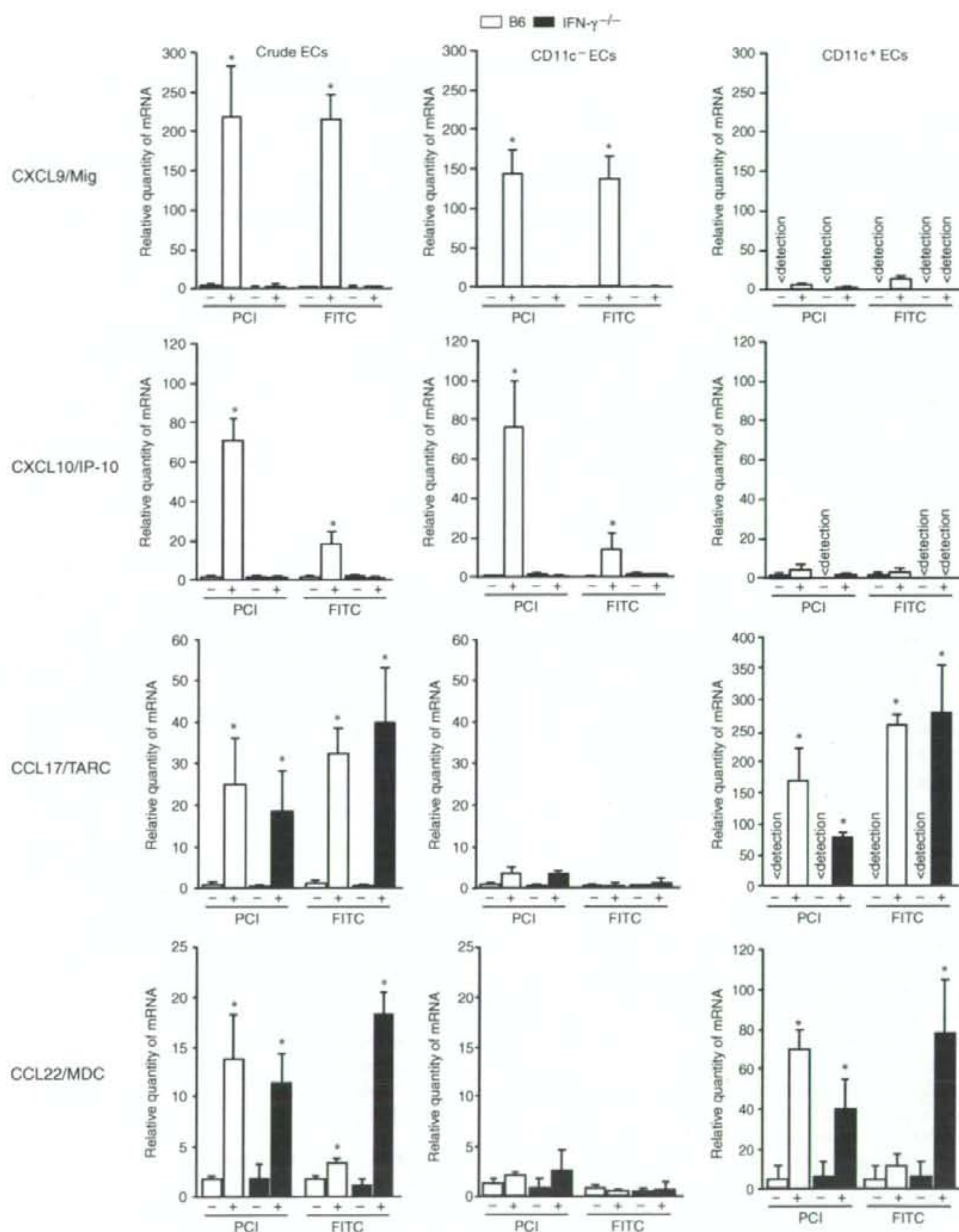

Figure 8. Real-time PCR analysis of mRNA expression for chemokines in ECs. B6 and IFN-$\gamma^{-/-}$ mice were sensitized with PCI and challenged once with PCI, or sensitized with FITC and challenged six times with FITC (twice a week for 3 weeks). Single-cell suspensions of epidermal cells were prepared from the challenged ears. Unfractionated (crude ECs), CD11c^{+} cell-depleted (CD11c^{-}), and CD11c^{+}-purified ECs were subjected to real-time PCR analysis for Th1 chemokines (CXCL9 and CXCL10) and Th2 chemokines (CCL17 and CCL22). The expression of mRNA is represented as fold increase ($2^{-\Delta\Delta C_t}$), where $\Delta\Delta C_t = |\Delta C_t(\text{sample})| - |\Delta C_t(\text{B6 CD11c}^- \text{ ECs without treatment})|$, and $\Delta C_t = [C_t(\text{sample})] - [C_t(\beta\text{-actin})]$. Data are expressed as the mean + SD of four mice. *$P<0.03$, compared between treated B6 mice or IFN-$\gamma^{-/-}$ mice, and no treatment B6 mice.

disappeared, but those of Th1 chemokines remained (Figure 8, middle). Therefore, it was considered that Th1 chemokines are produced by keratinocytes, whereas Th2 chemokines are derived mainly from LCs.

The expression of Th2 chemokines, but not Th1 chemokines, in LCs was confirmed with $CD11c^+$ cell-purified ECs. LCs from FITC or even PCl-challenged mice expressed Th2 chemokines but not Th1 chemokines (Figure 8, right). The results indicated that IFN-γ is required for keratinocyte expression of Th1 chemokines, but suppressive for LC expression of Th2 chemokines. IFN-$\gamma^{-/-}$ mice had the decreased ability of keratinocytes to produce Th1 chemokines even by PCl challenge, but retained the ability of LCs to produce Th2 chemokines particularly upon repeated FITC challenge.

DISCUSSION

In this study, we chose two distinct experimental systems to discriminate Th1 and Th2 cutaneous hypersensitivity reactions. The ordinary delayed-type CHS to PCl was used as a Th1-mediated response, and the immediate or late-phase reaction to repeatedly challenged FITC was used as a Th2-mediated response. We investigated the differences between the wild-type and IFN-$\gamma^{-/-}$ mice in these two cutaneous hypersensitivity systems. IFN-$\gamma^{-/-}$ mice were low responders in DTH to PCl, but high responders in the immediate and late-phase responses to FITC. The Th1/Th2 dichotomy-related immunological status was confirmed by the IFN-γ expression in PCl-challenged wild-type mice and the IL-4 expression in FITC-challenged IFN-$\gamma^{-/-}$ mice.

The depression of DTH to PCl and the enhancement of immediate and late-phase responses to FITC in IFN-$\gamma^{-/-}$ mice are not particularly surprising, because the Th2-preponderant immunological balance in these mice is easily expected as a result of a relief from suppression of Th2 cells by IFN-γ. In addition, participation of IFN-γ in both afferent and efferent limbs duly results in depressed DTH. However, as inflammatory cells did not infiltrate in the PCl-challenged ears of IFN-$\gamma^{-/-}$ mice, the observed downmodulation of cutaneous DTH could not be simply attributable to the systemic immunological shift to Th2 cells or to the impaired antigen-presenting function of cutaneous dendritic cells, but the failure in infiltration of T cells to the skin might be causative. Conversely, the extent of inflammatory cell infiltrate in FITC-challenged IFN-$\gamma^{-/-}$ mice was high. Therefore, chemokines produced by ECs appeared to be critical for determining the magnitude of these cutaneous responses.

Among ECs, keratinocytes and LCs are the essential sources of chemokines (Sebastiani *et al.*, 2002). $CXCR3^+$ Th1 cells infiltrated in the PCl-challenged B6 mice, whereas $CCR4^+$ Th2 cells were seen in the FITC-challenged IFN-$\gamma^{-/-}$ mice. Our real-time PCR analysis of EC-derived chemokines revealed that Th1 chemokines, CXCL9 and CXCL10, are produced by keratinocytes, and Th2 chemokines, CCL17 and CCL22, are mainly exposed by LCs. In IFN-$\gamma^{-/-}$ mice, the Th1 chemokines were not expressed by PCl challenge, whereas the expression of Th2 chemokines retained when challenged with PCl or FITC. Therefore, it is likely that IFN-γ is necessary for the production of Th1 chemokines by keratinocytes, but unnecessary or rather suppressive for the elaboration of Th2 chemokines by LCs. It is considered that the lack of development of cutaneous DTH to PCl in IFN-$\gamma^{-/-}$ mice stems from the inability of keratinocytes to produce Th1 chemokines. In accordance with this finding, our transfer study using immune lymph node cells obtained from sensitized B6 mice and labeled with CFSE and those from sensitized GFP mice demonstrated low infiltration of transferred lymphocytes in IFN-$\gamma^{-/-}$ mice.

The role of IFN-γ for the production of CXCL9 and CXCL10 by keratinocytes has been reported (Albanesi *et al.*, 2000b; Mahalingam *et al.*, 2001; Sebastiani *et al.*, 2002). CCL17 and CCL22 have also been reported to be released from keratinocytes (Sebastiani *et al.*, 2002), and a combination of IFN-γ and TNF-α is one of the most effective stimuli for keratinocytes to produce CCL17 and CCL22 (Kobayashi *et al.*, 2004). However, this study demonstrated that keratinocytes are not the main source of these Th2 chemokines, but that LCs are the producers. In accordance with this notion, cultured normal human keratinocytes cannot produce CCL17 even under stimulation with IFN-γ and TNF-α (Tsuda *et al.*, 2003). It has been reported that LCs produce or express various chemokines, including CCL17, CCL22, CXCL11, and CCL5 (Fujita *et al.*, 2004, 2005). Cytokines such as TNF-α, GM-CSF, IL-1β, and IL-4 stimulate LCs to produce CCL17 and CCL22 (Soumelis *et al.*, 2002). It is notable that IFN-γ is not a requirement for LCs to produce CCL17 and CCL22 but rather suppressive for their production (Xiao *et al.*, 2003). In IFN-$\gamma^{-/-}$ mice, therefore, Th2 cells are prone to infiltrate in the dermis, and the late-phase reaction can occur as a result of successful migration of Th2 cells to the skin. In this context, it is an interesting issue that the infiltrating lymphocytes showed epidermotropism possibly because of Th2 chemokine production by LCs. The production of the chemokines by LCs may support the recent concept that LCs are rather regulatory for the development of CHS (Kaplan *et al.*, 2005; Kissenpfennig *et al.*, 2005).

Interferon-γ, as well as IL-12 (Wolf *et al.*, 1994), is the key cytokine for Th1 responses. Furthermore, recent accumulating evidence has suggested the important role of IL-17 for CHS (Nakae *et al.*, 2002; He *et al.*, 2006). As IL-17 stimulates keratinocytes to release certain cytokines and chemokines with synergistic or additive effects when used together in IFN-γ (Albanesi *et al.*, 2000a), our results in IFN-$\gamma^{-/-}$ mice possibly stem from the relief of the synergistic effects. Our study suggests that IFN-γ has a mandatory role for conditioning the skin milieu by modulating Th1 and Th2 chemokines.

MATERIALS AND METHODS

Animals and chemicals

Eight to 10-week-old female B6 mice were purchased from Japan SLC (Hamamatsu, Japan) and Kyudo Co. (Tosu, Japan). B6 and IFN-$\gamma^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, ME), and GFP mice were obtained from Japan SLC. These mice were maintained in the Laboratory Animal Research Center in University of Occupational and Environmental Health under specific pathogen-free conditions. Female mice were used in this study. All animal experiments were performed according to the guidelines for the care

and use of animals approved by our university. PCl was purchased from Tokyo Kasei (Tokyo, Japan), FITC from Sigma Chemical Co. (St Louis, MO), and DNFB from Nakalai Tesque (Kyoto, Japan).

Sensitization and elicitation of CHS

For induction of CHS to PCl, mice were painted on the clipped abdomen with 50 μl of 5% PCl in ethanol/acetone (3:1). Five days after sensitization, mice were challenged by painting both sides of each ear with 20 μl of 0.5% PCl in ethanol/acetone (3:1). The ear thickness of mice was measured using dial thickness gauge (Ozaki Co., Tokyo, Japan) before and 24 hours after challenge. Ear swelling was calculated as (ear thickness after challenge)–(ear thickness before challenge).

For induction of CHS to FITC, mice were painted on the clipped abdomen with 200 μl of 1% FITC in acetone/di-*n*-butylphthalate (1:1). Five days after sensitization, mice were challenged by painting both sides of each ear with 20 μl of 0.5% FITC in acetone/di-*n*-butylphthalate (1:1) twice a week for 3 weeks. The ear thickness of mice was measured using dial thickness gauge before, 0, 1, 4, and 24 hours after challenge.

For induction of CHS to DNFB, mice were painted on the clipped abdomen with 50 μl of 0.5% DNFB in acetone/olive oil (4:1). The ears were challenged with 20 μl of 0.3% DNFB in acetone/olive oil (4:1).

Preparation of ECs, $CD11c^+$ cell-depleted ECs, purified $CD11c^+$ ECs, and lymph node cells

Ears were obtained from mice 24 hours after the last challenge with PCl or FITC, and epidermal and dermal skin was obtained by removing cartilage and subcutaneous tissues. After incubation for 1 hour at 37 °C in a 0.2% solution of trypsin in PBS, the epidermis was separated from the dermis, and ECs were dispersed in PBS supplemented with 10% fetal calf serum (Gibco, Carlsbad, CA) by rubbing the separated epidermal sheets (Tokura *et al.*, 1994). The cells were filtered through a cell strainer and washed twice in PBS. In some experiments, $CD11c^+$ ECs were purified with automagnetic-activated cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). After ECs were incubated with RPMI 1640 supplemented with 10% fetal calf serum for 30 minutes, ECs were first reacted with CD11c microbeads (120-000-322; Miltenyi Biotec) for 30 minutes and selected for $CD11c^+$ ECs, representing LCs. The purity of $CD11c^+$ cells was 70–80% (Sugita *et al.*, 2007). After removing $CD11c^+$ cells, $CD11c^-$ ECs were obtained and $CD11c^+$ cells were less than 0.1%. Among CD11c-positive fractionated cells from ECs, more than 99% of $CD11c^+$ cells were Langerin (eBioscience, San Diego, CA) positive (data not shown).

B6 and IFN-$\gamma^{-/-}$ mice were sensitized with 5% PCl and challenged once with 0.5% PCl, or sensitized with 1% FITC and challenged six times with 0.5% FITC (twice a week for 3 weeks). Single-cell suspensions of immune cervical lymph node cells were prepared from the treated mice.

Histological assessment

Twenty-four hours after challenge with PCl and FITC, mice were killed by cervical dislocation and ears were amputated. The ears were fixed with formalin and embedded in paraffin. Two- to three-micrometer- thick sections were cut and stained with hematoxylin and eosin. An indirect immunohistochemical staining was performed. The first antibodies included anti-CD3 (clone KT3, rat IgG2a; Chemicon, Billerica, MA), anti-IgE (clone 23G3, rat $IgG1_{\kappa}$; eBioscience), and IFN-γ (clone XMG1.2, rat $IgG1_{\kappa}$; eBioscience) and mAbs. A goat anti-rat IgG antibody conjugated with horseradish peroxidase was used as the second antibody, and reaction was visualized with diaminobenzidine.

Adoptive transfer

B6 mice were sensitized with 50 μl of 5% PCl in ethanol/acetone (3:1) on the clipped abdomen. Five days after sensitization, the axillary, bradial, and inguinal lymph nodes were removed, single-cell suspensions were prepared and labeled with 6.5 μM CFSE (Molecular Probes, Eugene, OR). The cells resuspended in RPMI containing 0.2% fetal calf serum were injected intravenously to naive B6 mice or naive IFN-$\gamma^{-/-}$ mice (5×10^6 cells per mouse). Immediately after the cell transfer, the ears were challenged with 20 μl of 0.5% PCl in ethanol/acetone (3:1), and the increase in ear swelling was measured 24 hours later. In parallel, ears of transferred B6 and IFN-$\gamma^{-/-}$ mice were histologically examined 24 hours after cell transfer. CFSE-positive cells in about 7 μm ear section of each mouse were counted.

In another transfer study, GFP mice were sensitized with 50 μl of 0.5% DNFB in acetone/olive oil (4:1). Five days later, the immune lymph node cells were prepared as above and injected intravenously to naive B6 mice or naive IFN-$\gamma^{-/-}$ mice (5×10^6 cells per mouse). The ears were challenged with 20 μl of 0.3% DNFB in acetone/olive oil (4:1). The ear swelling response, histology, and GFP-positive cells were examined as above.

Real-time quantitative PCR

Total cellular RNA was extracted with an RNA extraction kit (Promega, Madison, WI) from freshly prepared skin samples. RNA was then reverse-transcribed and amplified by random hexamer in single-tube assay using the TaqMan Reverse Transcription Reagants (Applied Biosystems, Foster City, CA) with gene-specific sense and antisense primers and a detection probe labeled on the 5′-end with the reporter dye 6-FAM. Primers and probes were obtained from TaqMan Gene Expression Assays Inventories (Applied Biosystems). Using an ABI Prism 7000 Sequence Detection Systems (Applied Biosystems), duplicate samples were reverse-transcribed and amplified under the following consecutive steps: 2 minutes at 50 °C, 10 minutes at 95 °C, followed by 50 amplification cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Sequence-specific amplification was detected as an increased fluorescent signal of 6-FAM exceeding the threshold limit during the amplification cycle. Quantification of gene-specific message levels was determined by comparing fluorescence intensity from unknown RNA samples to the fluorescence intensity of standard curve generated from control mRNA levels. Amplification of the gene for mouse β-actin was performed on all samples to control interspecimen variations in RNA amounts. The result for each gene was normalized to the quantity of mouse β-actin detected in the sample. Levels of gene-specific message were graphed as normalized message units as determined from standard curve.

Statistical analysis

Data were analyzed using an unpaired two-tailed *t*-test. $P < 0.05$ was considered to be significant.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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CONTACT DERMATITIS

Percutaneous penetration *via* hand eczema is the major accelerating factor for systemic absorption of toluene and xylene during car spray painting

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Background: For absorption of organic solvents, the respiratory tract is well known as the major site, but percutaneous absorption might be critical in some workplaces.

Objectives: The aim of the study is to determine whether the skin, if disordered, is 1 of the major routes of organic solvent absorption.

Patients/Methods: 72 male workers who painted the car body in the booth of a Japanese car company were participated in this study. The severity of hand eczema, urinary metabolites of organic solvents and the concentration of airborne organic solvents were measured.

Results: The correlation coefficiency between the skin severity index and the urinary concentration of hippuric acid or methylhippuric acid was statistically significant. There was no significant correlation between their urinary values and the air concentration of mixed organic solvents.

Conclusions: The skin is a more critical absorption route for organic solvents than the respiratory tract in some occupational settings. Hand eczema is a common disease and has a possibility to be a critical absorption route of organic solvents.

Key words: hand eczema; hippuric acid; methylhippuric acid; toluene; xylene. © Blackwell Munksgaard, 2008.

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It is well known that exposure to a low or moderate concentration of organic solvents may cause various symptoms such as headache and dizziness (1, 2). The health risk is assessed by biological monitoring of exposure through the evaluation of the internal dose. The threshold limit values (TLVs) are guidelines designed for use by industrial hygienists in making decisions regarding safe levels of exposure to various chemical substances and physical agents found in the workplace. Toluene and xylene are used in various workplaces, in particular industrial settings. When toluene and xylene are absorbed into the body, they are discharged in urine after several steps of chemical changes (3, 4). Urinary hippuric acid and methylhippuric acid are the most frequently used biomarkers in the biological monitoring of occupational exposure to toluene and xylene (5). There have been data of urinary hippuric acid in 3,947 workers and of methylhippuric acid in 1,523 workers in Japan (5). The results can be classified into 3 levels, and the category 3 represents the urinary levels of metabolites of organic solvents higher than the classified American Conference of Governmental Industrial Hygienists (ACGIH) recommendations of TLVs and the biological exposure indices issued in 1988–1989.

In addition to the respiratory intake, the percutaneous absorption is expected to be an important factor of the biological exposure to organic solvents, but the skin has not been estimated as a critical route to determine the TLVs. Only a couple of chemicals are known to be absorbed through the skin, as represented by *N,N*-dimethylformamide (6). While normal skin may be resistant to absorption of organic solvents (7), it is possible that pathological or injured skin allows the solvents to penetrate the skin. In this respect, hand eczema

is thought to be the most critical skin disorder, because its incidence is very high (8) and the hand is easily exposed to the solvents (9). Moreover, hand eczema often occurs in patients with atopic dermatitis or atopic backgrounds (10, 11). A recent finding has suggested that a high incidence of mutation of filaggrin is seen in atopic patients (12). Because filaggrin deeply participates in the skin barrier function, individuals with the disordered barrier are prone to have atopic dermatitis as a result of feasible skin penetration of exogenous substances.

To investigate the influence of hand eczema on the absorption of organic solvents, we monitored the levels of urinary hippuric acid and methylhippuric acid in relation to the severity of hand eczema in spray painters of an automobile plant.

Materials and Methods

72 male workers who painted car body for 8 hrs a day in the painting booth of a Japanese car company located in Kosai City, Japan, were enrolled in this study. They worked in 7 different, 65×6 m-wide booths equipped with general ventilation system. All the workers wore Tyvek dustproof overalls, gas masks that are suitable for Japanese Industrial Standards, and nylon electrostatic gloves. The concentrations of organic solvents in air were lower than their respective TLVs of ACGIH recommendation in all painting booths. For biological monitoring of toluene and xylene, urine hippuric acid and methylhippuric acid were measured, respectively. Urine samples were collected on the last day of the working week. To avoid the influence of benzoic acid, aspirins or food preservatives are prohibited for 1 week. The concentrations of hippuric acid and methylhippuric acid were measured by high performance liquid chromatography (Enshu Medical Laboratory, Hamamatsu, Japan). The lowest detection levels of these metabolites were 0.01 g/l. Urine hippuric acid (mean $\pm$ SD $= 0.3375 \pm 0.3283$ g/l) and methylhippuric acid (mean $\pm$ SD $= 0.0436 \pm 0.0346$ g/l) levels belonged to the category 1 or 2 in all the workers, and thus satisfied the ACGIH issued recommendations. Samples of air were collected with a 1 l vacuumed glass sampling tube. The levels of toluene and xylene were quantified by gas chromatography and mass spectrometry (Department of Experiment and Analysis, Suzuki Motor Company, Hamamatsu, Japan).

The severity of hand eczema was evaluated by a dermatologist in dryness, erythema, scaling, dyshidrosis, fissuring, and lichenification using simple visual scoring: 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The skin severity index was represented by the sum of these values (13). To explore the association of the urinary metabolites of organic solvents with the skin severity index, Spearman's correlation coefficients were used. All statistical analyses were performed with JMP V5.0.1™.

Results

As shown in Fig. 1, the urine hippuric acid level of 72 spray painters (mean $\pm$ SD $= 0.3375 \pm 0.283$ g/l) was significantly higher than that of healthy 41 male workers who had not used organic solvents (mean $\pm$ SD $= 0.1671 \pm 0.2243$ g/l) ($P = 0.0038$). The urine methylhippuric acid level of 41 normal subjects was under the lowest detection level. The skin severity index in the workers ranged from 0 to 6 points. The correlation coefficiency between the skin severity index and the urinary concentration of hippuric acid or methylhippuric acid was statistically significant (Fig. 2). In particular, a high correlation coefficiency (0.6099) was observed in hippuric acid. It was noted that workers without any hand eczema (the skin severity index, 0) exhibited extremely low levels of urinary hippuric acid, except for 1 worker. All the workers wore a gas mask that was regularly maintained, and the

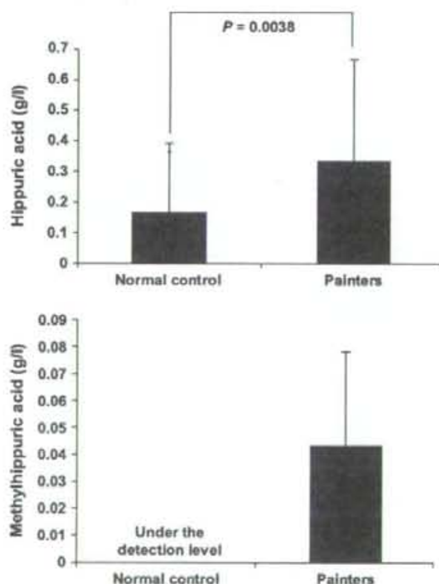

Fig. 1. Urinary metabolites of toluene and xylene of 72 spray painters are significantly higher than those of 41 normal subjects who have not been used organic solvents.

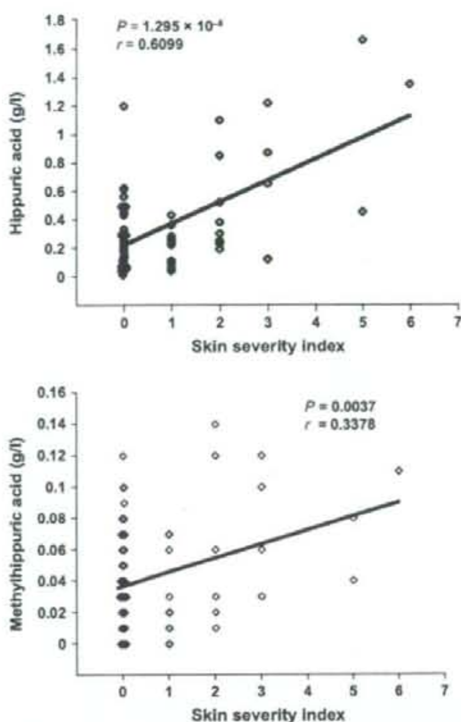

Fig. 2. Positive correlation between urinary hippuric acid (top) or methylhippuric acid (bottom) and skin severity index of hand eczema.

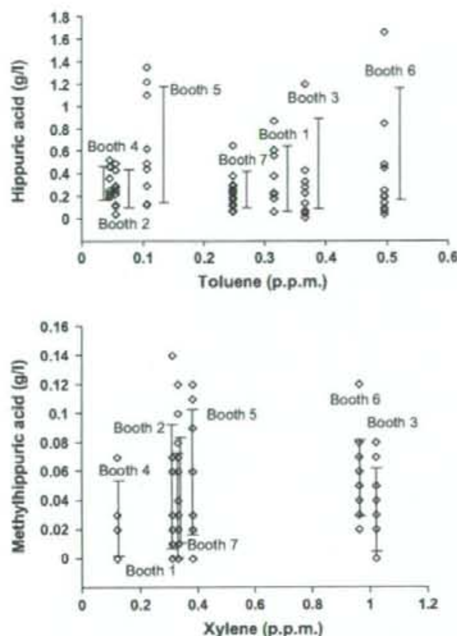

Fig. 3. Absence of correlation between urinary hippuric acid and toluene concentration in air (top) or methylhippuric acid and xylene concentration in air (bottom). The concentrations of toluene and xylene in air were measured in the 7 different booths and correlated with the concentrations of urinary hippuric and methylhippuric acids of the workers in each booths. Error bar represents the mean ± SD of urinary hippuric acid or methylhippuric acid levels of painters in each booths.

air exchange was efficiently performed in the 7 booths. To further exclude the possible absorption through the respiratory tract, we also examined the relationship between the urinary concentration of hippuric acid or methylhippuric acid, and the air concentration of toluene and xylene, respectively. Figure 3 shows these correlations in 7 different booths. There was no significant correlation between the air levels of toluene and xylene and urine concentrations of hippuric acid and methylhippuric acid, respectively, confirming that the respiratory absorption of organic solvents did not contribute to the urinary levels in this workplace. These data indicate that the concentrations of urinary hippuric acid and methylhippuric acid depend on the severity of hand eczema.

Discussion

Our study clearly showed that workers with hand eczema have high urinary levels of hippuric acid and methylhippuric acid when occupationally exposed to organic solvents. Although the respiratory tract has been estimated as the major absorption system of the solvents (3, 4), we found that the urinary concentration of hippuric acid or methylhippuric acid did not depend on the air concentration of mixed organic solvents in our occupational settings. Thus, the skin is 1 of the crucial absorption routes for organic solvents.

Skin protection items such as protective gloves and barrier creams are necessary for prevention from the irritation by or penetration of organic solvents (13). The workers in our study wore nylon electrostatic gloves, but they could not protect the penetration of organic solvents. The workers also used emollients for skin care with insufficient protection. Our findings indicate that the skin protection from organic solvents is difficult. The actual advantage of barrier creams over emollients for skin protection is still debated (13).

Hand eczema is a common disease and induced by organic solvents themselves as well as atopic background (10, 11). Given that organic solvents can disrupt the skin barrier by removing ceramide of the stratum corneum (14), the induced acute

barrier disruption may further exaggerate the percutaneous absorption of the solvents. It should be kept in mind that the absorption *via* eczematous skin is the important mechanism underlying systemic absorption of organic solvents when workers deal with the solvents by hands.

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アトピー性皮膚炎への奏効機序からみたNarrowband UVBとPUVA療法のケラチノサイトに対する影響

戸倉　新樹

Narrowband UVB（NB-UVB）とPUVA療法はどちらもアトピー性皮膚炎の治療に用いられる。ターゲットとなる細胞には，ケラチノサイト，Langerhans細胞（LC）・真皮樹状細胞（dDC），T細胞，肥満細胞，C線維があるがケラチノサイトは最も重要なものである。NB-UVBはケラチノサイトの前炎症性サイトカイン産生をあまり高めずに，Th2ケモカイン産生を抑制し，ADに適した治療ということができる。また酸化的損傷の程度も低く，安全性にも優れる。PUVA療法は前炎症性サイトカインの産生を抑制し，さらに言えば全てのサイトカイン・ケモカイン産生を抑制すると推察される。

（皮膚の科学，増*9*：*6*－*10*，*2007*）

キーワード：*narrowband UVB*，*PUVA*，ケラチノサイト，ケモカイン

1．はじめに

光線療法は種々の皮膚疾患に対して行われるが，アトピー性皮膚炎（AD）もそうした疾患に含まれる。特に難治性のADは照射操置さえあれば，よい適応となる。通常，narrowband UVB（NB-UVB）かソラレン（psoralen）＋UVA（PUVA）が光線療法として用いられるが，最近は簡便さもあってNB-UVBの頻度が増している。これらの治療は元来，乾癬に対して使用されたものであり，従ってADへの奏効機序は乾癬と同様なものと異なるものとが存在する。

ここではADに対する奏効機序を念頭に置きながら，NB-UVBとPUVAの表皮角化細胞（ケラチノサイト）に及ぼす影響を考えてみたい。

2．ADを念頭においたNB-UVBとPUVAのターゲットとその影響の鳥瞰

ADはフィラグリンなどが構成するバリアの異常があってアレルギーを起こす疾患と考えられている[1]。ADでは，ケラチノサイト，Langerhans細胞（LC）・真皮樹状細胞（dDC），T細胞，肥満細胞，C線維などの構成要素が皮膚病変形成に関わっている（**Fig. 1**）。これら個々の細胞がNB-UVBやPUVAによりどのように変調され，それが治療効果に繋がっているかを探るのが本稿の目的となる。

1）ケラチノサイト

NB-UVBとPUVAはともにケラチノサイトの増殖を抑制する，サイトカイン・ケモカインの産生は次項に述べるように両者で異なる。プロスタグランディンE2の産生はUVBで亢進する。

2）LC・dDC

UVBはLCの数を減少させる。そうした皮膚にハプテンの感作を行うと接触過敏症の成立は抑制される。これは1970年代から1980年代にかけて「local low-dose UVB-induced immunosuppression」として知られ，メカニズムに関し多くの研究が為されてきた。結局は制御性T細胞（regulatory T cell）誘導という現在の免疫学の言葉で置き換えられているが[2]，その機序は依然として判然としない。最近は接触過敏症において，LCは制御性（抑制性）でありdDCは促進性であるという研究結果が3つの研究グループから発表されている[3]。このことを踏まえて，UVBが本当にLCの抗原提示能を抑制するのか，実はdDCの抗原提示能を抑制していたのか，研究成果が待たれる。

3）T細胞

UVBもPUVAも増殖については抑制性である。サイトカイン産生についてはPUVAにおいて特異な面がみられる。PUVA処理した末梢血リンパ球はTh1サイトカインの産生が高まり，Th2サイトカイン産生は減少する。こ

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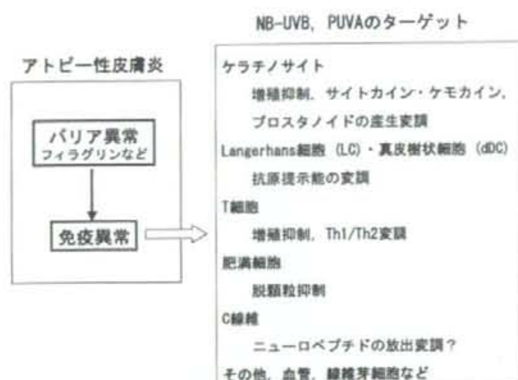

Fig.1 アトピー性皮膚炎におけるNB-UVB，PUVAのターゲット

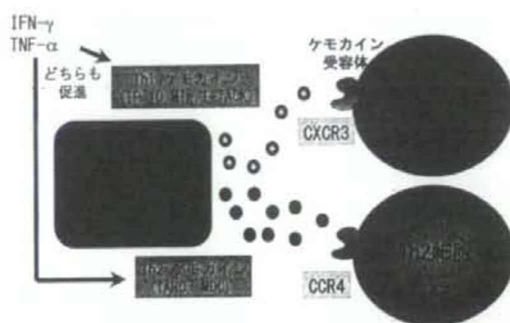

Fig.2 皮膚におけるケモカインおよびケモカイン受容体

Fig.3 ケモカインへの影響による疾患選択

れについては後述したい。

4）肥満細胞

PUVAが肥満細胞の脱顆粒を抑制する観察が為されている。

5）C線維

ニューロペプチドの放出変調などが起こるか注目される。

以上の中から，ここでは我々の行った研究結果として，NB-UVBとPUVAのケラチノサイトに対する影響を紹介したい。

3．NB-UVBのケラチノサイトへの影響

1）ケラチノサイトのケモカイン・サイトカイン産生への影響

ケラチノサイトがいかなるケモカインを産生するかということは，皮膚に集積するT細胞を決定することになる。ケラチノサイトはTh1/Tc1細胞を引き寄せるTh1ケモカインと，Th2細胞を引き寄せるTh2ケモカインを産生する。Th1ケモカインの代表的なものはIP-10，Mig，I-TACKであり，Th2ケモカインの代表的なものはTARC，MDCである。つまりケラチノサイトは両T細胞サブセットに対するケモカインを共に産生する（**Fig. 2**）。TARCについては注意を要する。実験によく使われるHaCaT細胞というケラチノサイトのcell lineではTARC産生があるが，培養正常ヒトケラチノサイトでは適切な刺激をしても産生が認められない。ADや皮膚T細胞リンパ腫のケラチノサイトでは免疫染色でTARCの存在が報告されている。こうした乖離の意味合いについては未だ不明である。

さてケラチノサイトにNB-UVBやPUVA処理して前炎症性サイトカインとTh1およびTh2ケモカインの産生への影響をみる。もしその処理がTh1ケモカイン産生を抑制する，あるいはTh2ケモカイン産生を促進するのであれば，乾癬に有効であろう。一方その処理がTh2ケモカイン産生を抑制する，あるいはTh1ケモカイン産生を促進するのであれば，ADに有効であるといえる（**Fig. 3**）。

NB-UVBの特性を調べるためには，当然，その対照としてBB-UVBと比較しなければならない。しかし両UVBは異なった波長であるために，どの照射量で比較すべきかが問題となる。**Table 1**にみられるように，最小紅斑量（MED）ベースにおいても治療照射量においても，NB-UVBはBB-UVBの8～10倍が相当量である。従ってBB-UVBの10倍量でNB-UVBを比較検討するのが適切と考えられる。

培養HaCaT細胞をNB-UVB（100，1000mJ/cm²）またはBB-UVB（10，100mJ/cm²）照射し，インターフェロン-γを添加して刺激し，48時間培養後の上清に対してELISA，細胞そのものに対してRT-PCRを行い，サイトカイン・ケモカインの産生・表出をみた。**Table 2**にその検討結果を示す[1]。BB-UVBはすでによく知られているように，IL-1α，TNF-αの産生を増強させる。これは

Table 1　BB-UVBとNB-UVBにおける同等効果照射量

BB－UVBとNB－UVBの照射量の比較（mJ/cm²）

	MED	治療域
BB-UVB	70-150	50-150
NB-UVB	500-1200	400-1200

Table 3　NB-UVBとPUVAのケラチノサイトへの影響

	DNAへの傷	酸化的DNA損傷	前炎症性サイトカイン産生	ケモカイン産生
NB-UVB	CPD形成	小	⇧(mild)	Th1⇧ Th2⇩
PUVA	8-MOP-DNA photoadduct 形成	大	⇩	Th1⇩ Th2⇩（多分）

Table 2　ケラチノサイトの前炎症性サイトカインと Th1/Th2ケモカイン産生に対するUVBの影響

	Proinflammatory cytokines		Th1 chemokines		Th2 chemokines	
	IL-1α	TNF-α	IP-10	Mig	MDC	TARC
BB-UVB	⇧⇧	⇧⇧	⇨	⇧	⇩	⇩⇩
NB-UVB	⇧	⇧	⇨	⇧	⇩	⇩⇩

両UVBとも前炎症性サイトカイン産生を増強。しかしNB-UVBは穏やか。
両UVBともTh1ケモカイン産生を増強。Th2ケモカイン産生を減弱。

NB-UVBでも同様であり，両サイトカインの産生は促進した。しかし，BB-UVBと比べ，この前炎症性サイトカインの産生増強作用はNB-UVBでは比較的弱かった。従って，NB-UVBは炎症をあまり惹起させることなく，治療効果をあげることができる。両UVBともTh1ケモカインであるMigの産生を増強させたが，IP-10については影響を与えなかった。一方，Th2ケモカインであるTARC，MDCの産生は抑制した。すなわち両者は同程度にTh1ケモカイン産生を亢進させ，Th2ケモカイン産生を抑制する。このことを確認するために，こうした培養上清を用いてtranswellケモタキシスアッセイを行ったところ，UVB照射ケラチノサイトの培養上清ではTh1のケモタキシスは亢進しTh2のケモタキシスが減弱していることが確認された[4]。

以上のように，NB-UVBは前炎症性サイトカイン産生をあまり高めずに，Th2ケモカイン産生を抑制し，ケモカイン・サイトカインという面からみれば，ADに適した治療ということができる。しかし，解釈には以下の点からの考慮が必要となる。第1にUVBは表皮内へのTh2細胞浸潤を抑制することができるが，真皮への浸潤は抑制できないかもしれない。第2に，Th1病である乾癬に奏効する理由はケモカインでは説明できない。第3に，現在，表皮の主なTh1ケモカインソースはケラチノサイト，Th2ケモカインソースはLCであることが判明している[5]。従って，UVBのLCへの影響も調べなければならない。これらは今後の問題であろう。

2）ケラチノサイトの酸化的損傷への影響

NB-UVBとBB-UVBの安全性を比較するのは困難であり，最終的には皮膚癌発生の差をみなければならないが，長期を要する調査であるため現時点では望めない。そこで両UVBのケラチノサイトにもたらす影響を，8-hydroxy-2'-deoxyguanosine（8-OH-dG）の生成量について検討した[6]。8-OH-dGによる突然変異誘発はG：C→T:A transversionであり，シクロブタン型ピリミジンダイマー（CPD）ほどではないが，発癌に関わるとされている。前述のBB-UVB:NB-UVB=1:10照射量で比較すると，NB-UVBの8-OH-dG生成は，BB-UVBのそれより低く酸化的損傷が小であることが確認された。因みにPUVAより低値であった。このことから直ちに結論を出すことはできないが，NB-UVBの低損傷性が示唆された。

4．PUVAのケラチノサイトサイトへの影響

1）ケラチノサイトでのソラレン-DNA光産物産生

ソラレンの最も重要なターゲット分子はDNAであり，UVA照射によりDNAに光結合する。ソラレンの中では8-メトキシソラレン（8-MOP）が専ら治療に使われ，UVAの働きでチミンに結合し，8-MOP-DNA monoadductを作る。UVAの照射量が多ければ2重鎖DNAのチミンを橋渡しして，cross linkを生成する。すなわちUVBはCPDを生成してDNAに傷を付けるように，PUVAもDNAに8-MOPによる傷を付ける。

PUVA処理された細胞が生きていれば，8-MOPは1日で約半数がDNA修復機構により排除される[7]。高カルシウム培地の方が低カルシウム培地より排除率が高い。またレチノイドを添加すると排除が遅延する。従ってRe-PUVA療法では8-MOPがDNAに結合した状態を維持しやすいといえる[8]。

2）ケラチノサイトのサイトカイン産生への影響

UVBがケラチノサイトの前炎症性サイトカイン産生を亢進させたのに対し，PUVAは抑制する[8]。これはIL-1αで確認されており，15ng/mlの8-MOPと1 J/cm² UVA処理でIL-1α産生は抑制される。因にこの処理はケラチノサイトにおいて0.52 photoadducts / 10^6 DNA塩基を生成する。内服PUVAにおける血中8-MOP濃度は約100 ng/mlであり，1 J/cm²以上のUVAは治療上用いられる照射量であるので，前炎症性サイトカインの産生抑制は，実際的に起こっていると考えられる[8]。

因みにT細胞をPUVA処理した時のサイトカイン産生はどうなるのであろうか。これは興味深い結果が得られており，Th1サイトカインは亢進し，Th2サイトカインは低下する[9,10]。従ってTh2病であるADや皮膚T細胞性リンパ腫にとって治療上有利となる。こうしたサイト

カインの変調が選択的に起こる理由は定かではないが，PUVA処理自体に特異性は求め難い。恐らくTh2細胞のサイトカイン産生の方が，Th1細胞のサイトカイン産生よりPUVAによって低下しやすく，そのために抑制から解かれたTh1細胞のサイトカイン産生が相対的に亢進するのではないかと考えられる。

5．おしまいに

Table 3にNB-UVBとPUVAのケラチノサイトへの影響をまとめる。どちらもADに有効であるが，ケラチノサイトに及ぼす一つひとつの事項は同じではない。特に前炎症性サイトカイン産生，ケモカイン産生については異なる。こうした違いがADのどのような病態に対して効果的か否か，ひいては皮膚炎の違いにより選択すべき光線療法があるのかということが今後の問題とされよう。

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6 ウイルス感染と薬剤性過敏症症候群（DIHS）

Drug-induced hypersensitivity syndrome
and virus infection

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Key words：DIHS，HHV-6，風疹，ウイルス，過敏症

Abstract

薬剤性過敏症症候群(DIHS)は薬疹とHHV-6の再活性化を伴う疾患である。この薬剤反応とウイルスの再活性化の機序は未だ明確には解明されていないが，薬疹が契機となりウイルスの再活性化が誘発されると解釈される。カルバマゼピン，フェニトイン，フェノバルビタール，ゾニサミド，それからジアフェニルスルホン，サラゾスルファピリジン，メキシレチン，アロプリノール，ミノサイクリンが原因薬として多く，肝障害，発熱，異型リンパ球出現などをみる。

はじめに

薬剤性過敏症症候群(drug-induced hypersensitivity syndrome, DIHS)は，薬疹であるがウイルスの再活性化でもある。1988年に同号のArchives of Dermatologyに杏林大皮膚科[1]と愛媛大皮膚科[2]から，重症型遷延性薬疹におけるhuman herpes virus-6 (HHV-6)の関与が示唆される論文が発表され，DIHSと命名されるに至った。このエポックメイキングな出来事を契機に，次々と多くの症例報告がなされ，典型例の供覧が反復される一方で，さまざまな点において，非典型的な症例も報告されるようになった。ここではまずDIHSについて一般的な事柄を概説し，次いで薬剤とウイルスの関わる疾患のメカニズムについて考えてみたいと思う。

1．DIHS典型例と非典型的事項

臨床像・組織像，原因薬剤，再活性化されるウイルス，合併症，検査値について典型的及び非典型的事項を記す。

1) 臨床像，組織像

臨床像について，初期は紅斑丘疹型あるいは多形紅斑型が多く，紅皮症になりやすい，というのが典型例である。顔面は腫脹し，眼周囲は皮疹が抜け，口周囲には好色丘疹，膿疱，小水疱，鱗屑が出現する（図1）。咽頭の発赤や口蓋の紫斑も出現することがある。38℃以上の発熱とリンパ節腫脹も伴うのが一般的である。

こうした典型的皮疹に加え，小膿疱を顕著に伴い acute generalized exanthematous pustulosis (AGEP)に似るタイプが報告され[3]，現在ではDIHSの一皮疹型として認知された感がある（図2）。DIHSはStevens-Johnson 症候群(SJS)や中毒性表皮融解壊死症(toxic epidermal necrolysis, TEN)といったgraft-versus-host disease (GVHD)型の薬疹形態はとりにくい，つまり表皮壊死がみられないということが言われてきた。しかし苔癬型組織反応を示し表皮壊死が顕著な例も稀には報告されている。

通常，皮疹を含めた臨床症状は再燃することがしばしばみられ，典型的には2峰性の皮疹や発熱をみる。2峰性目の時に肝機能障害が起こることもある。

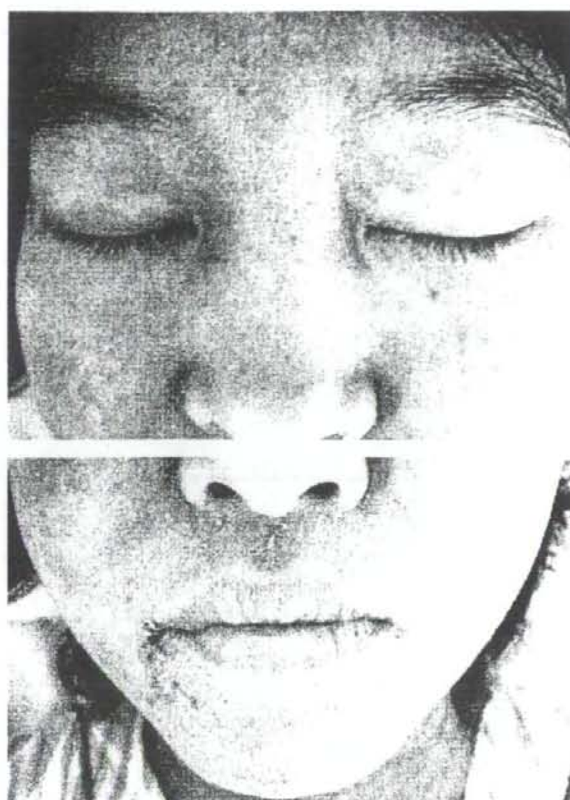

図1　DIHSの顔面の皮疹

2) 原因薬剤

DIHSは比較的限られた薬剤で起こることがいわれてきた。抗けいれん薬であるカルバマゼピン，フェニトイン，フェノバルビタール，ゾニサミド，それからジアフェニルスルホン(DDS)，サラゾスルファピリジン，メキシレチン，アロプリノール，ミノサイクリンの9薬剤である[4]。しかしその後，シアナミド，コデイン，クロミプロミン，トラピジルなどの非典型的な薬剤，さらには健康食品であるスピルリナ[5]も報告されている。軽度のDIHSを含めると，今後さらに種類の増加が予見される。従って，被疑薬を挙げる場合，多剤を内服しているときには，典型的な薬剤をまず考えるが，典型的な薬剤を内服していなくても，DIHSを否定できない。通常，内服開始後2～6週間（平均4週間）で発症し，原因薬中止後も2週間以上遷延する。

3) HHV-6以外のウイルスの再活性化

DIHSは基本的にHHV-6が再活性化する薬疹であるが，これ以外のウイルスが再活性化することも明らかになっている。とくにサイトメガロウイルス(CMV)の再活性化は多くの症例で認められ[6]，肝障害の程度との関連がいわれている（図3）。加えてHHV-7，EBウイルスも報告されている。これらはすべてヘルペスウイルスであり，他の皮膚疾患と同様，いかにヘルペスウイルスが皮膚病変発現に関わっているかを思い知らされる。

4) 合併症

合併症の種類についても報告が増加している。腎障害，脳炎など中枢神経障害，肺炎（図3），甲状腺炎，心筋炎，I型糖尿病などが，

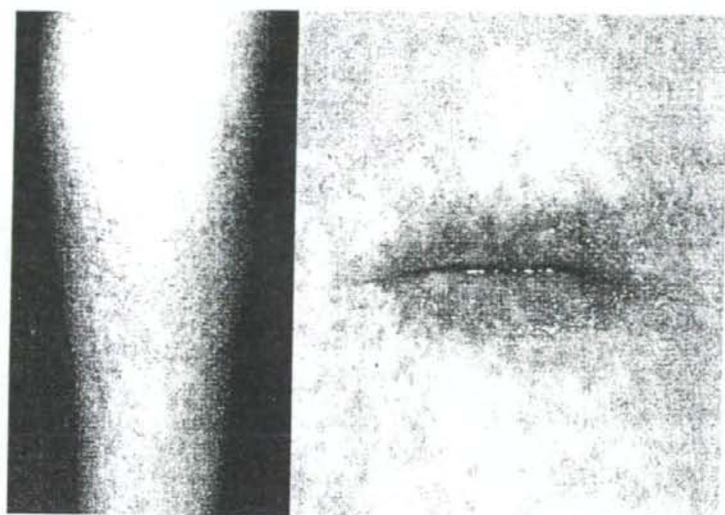

図2　AGEPの形態をとるDIHS例

図3　HHV-6とCMVの再活性化を伴い高度の呼吸器不全，肝障害を起こしたDIHS例

DIHSの提唱後に注目を集めている。今後も思わぬ合併症が見出されることも予想される。

5) 検査値

通常，肝機能障害があり，白血球増多（11000/mm³以上），異型リンパ球（5%以上），好酸球増多（1500/mm³以上）のうちいずれかがみられる。HHV-6の再活性化は，ペア血清でHHV-6 IgGの抗体価が4倍以上の上昇であることが必要となる。ペア血清は発症後14日以内と28日以降の2点行うと確実である。免疫グロブリンの低下も報告され[7]，これが病態に

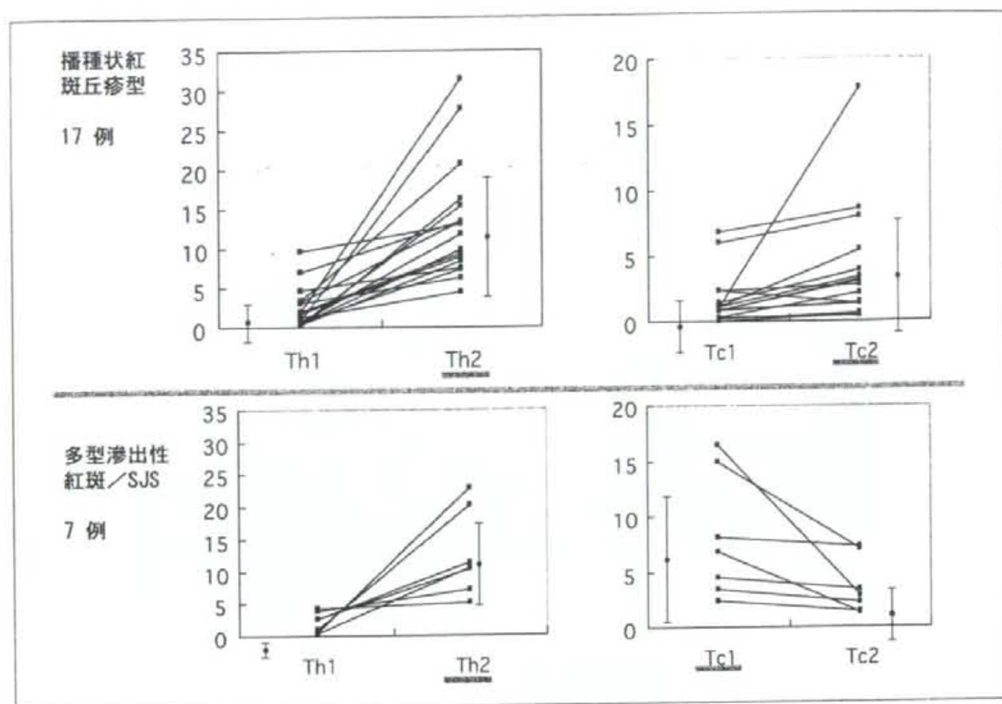

図4　薬疹の末梢血T細胞サブセット

どのような影響を与えるかについても考察されている。

2．HHV6再活性化の機序

薬剤に対するアレルギーで始まるこの疾患が，HHV-6などの再活性化を導くのは非常に興味深い。薬剤反応とウイルス再活性化の順序において，第1イベントとして薬剤に対する反応，第2イベントとしてHHV-6 の再活性化，というのが当然の見方であろう。これを支持するいくつかの現象をあげてみたい。

第1に，好酸球増多に引き続いて2相目の反応，すなわち皮疹の再燃や肝酵素の上昇がみられる症例がある。これは薬剤の反応に引き続いて，ウイルスに対する反応が起こることを示唆する。

第2に，薬疹患者の末梢血リンパ球のサブセットであるTh1(CD4+CXCR3+)，Th2(CD4+CCR4+)，Tc1(CD8+CXCR3+)，Tc2(CD8+CCR4+)を経時的に追ってみる。播種状紅斑丘疹型ではTh2とTc2の増加があり，多形紅斑型/SJS型の薬疹ではTh2とTc1の増加がある（図4）[8]。これに対しDIHSでは，最初にCD4陽性T細胞の活性化が起こり，次にCD8陽性T細胞の活性化が起こる（図5）。これを薬剤反応性CD4陽性T細胞の活性化に続いて，ウイルス感染細胞に対するCD8陽性T細胞の活性化が起こっていると読み解くこともできる。さらにDIHSの臨床経過ではTh2/Tc2優位の状態がTh1/Tc1優位の状態に変化する（図6）[8]。

第3に，皮疹の組織像についても，第1相目がCD4陽性T細胞の浸潤が優位にみられ，第2相目の皮疹ではCD8陽性T細胞浸潤が優位になる例もある。

播種状紅斑丘疹型や多形紅斑型/SJS型の薬疹

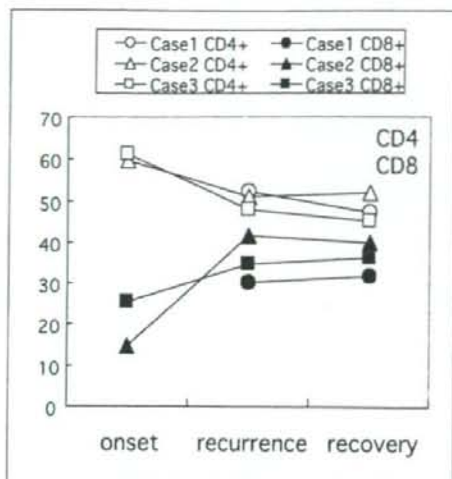

図5．DIHSにおけるCD4+細胞(Th)とCD8+細胞(Tc)の経過による変化

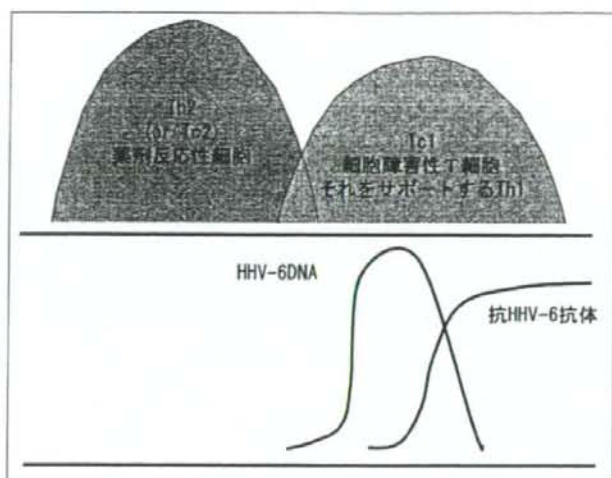

図6．DIHSにおけるT細胞サブセットの変化

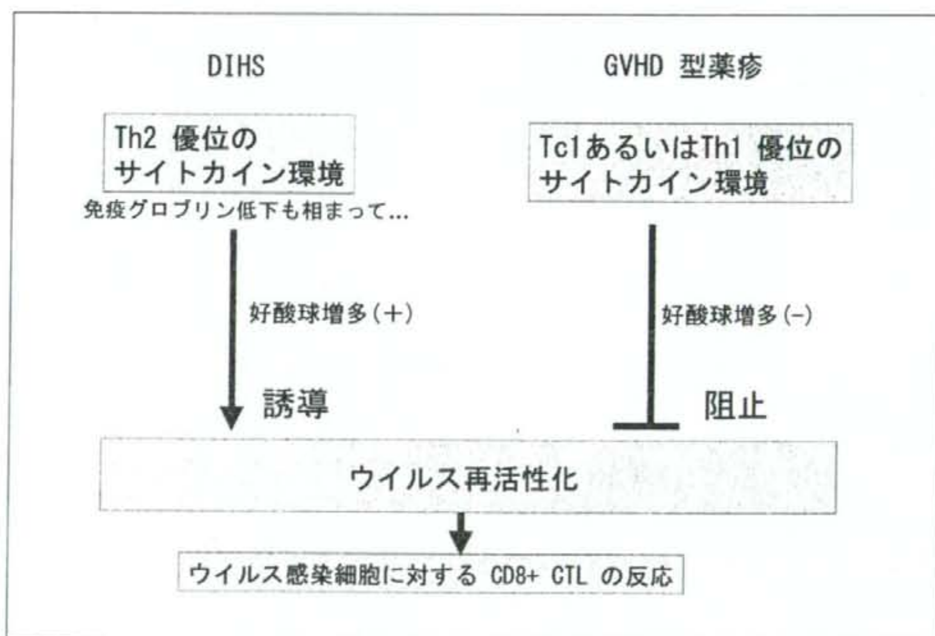

図7　薬疹型とウイルスの再活性化の関係