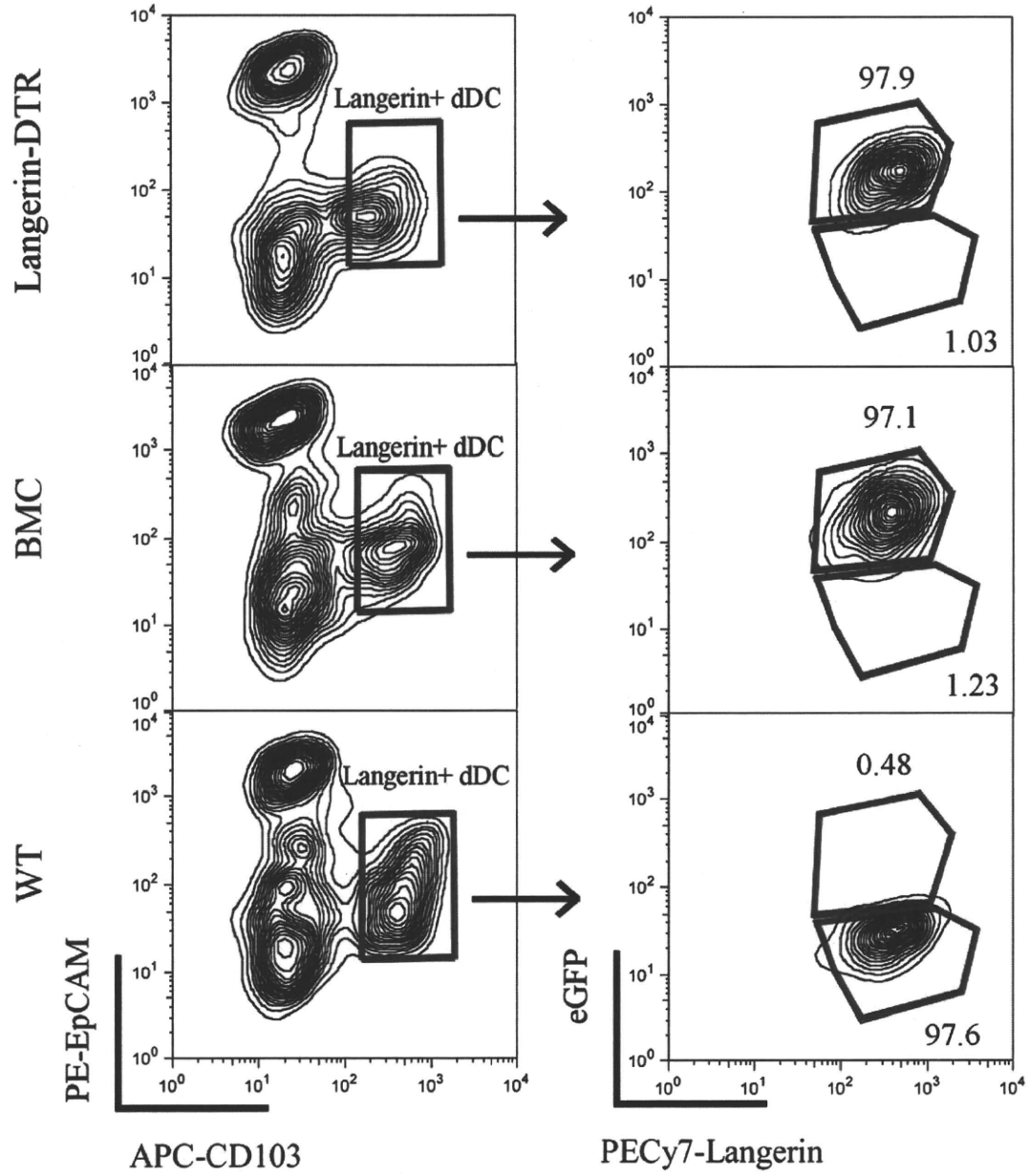

FIG E1. Chimerism of langerin-positive dDCs in BMC mice. The CD11c$^+$ MHC class II$^+$ EpCAM$^-$ CD103$^+$ langerin-positive DCs of the dermis from langerin-eGFP-DTR, BMC, or B6 mice *(left panels)* were analyzed by means of flow cytometry for the expression of GFP. The frequencies (percentages) of GFP$^+$ and GFP$^-$ cells among langerin-positive dDCs are shown *(right panels)*. *APC*, Allophycocyanin; *PE*, phycoerythrin; *PECy7*, phycoerythrin-Cy7; *WT*, Wild Type.

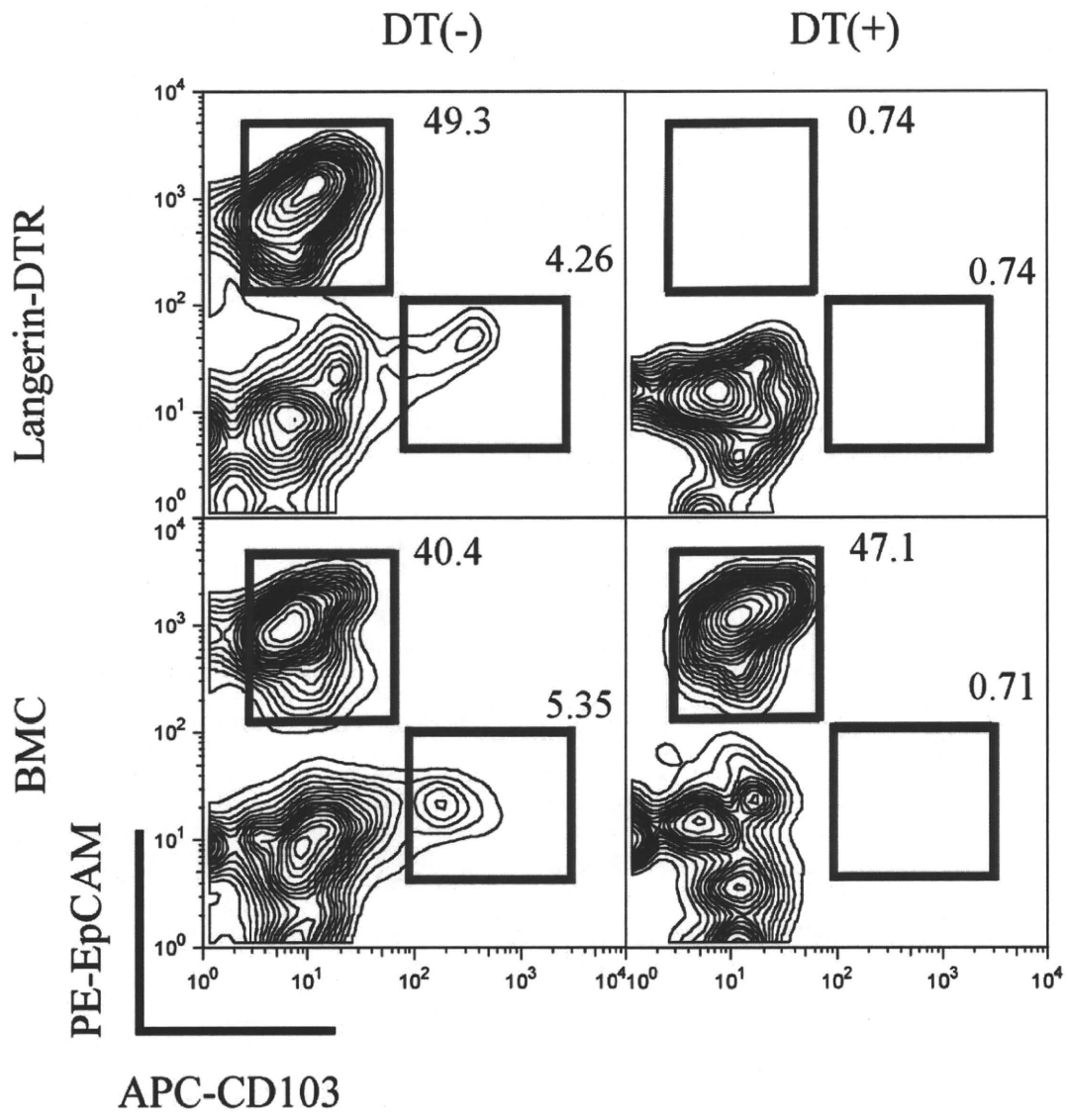

FIG E2. Effect of DT on reconstituted langerin-positive dDCs in BMC mice. Langerin-eGFP-DTR and BMC mice were treated with or without DT, and the $CD11c^+$ MHC class II^+ $EpCAM^-$ $CD103^+$ and $CD11c^+$ MHC class II^+ $EpCAM^+$ $CD103^-$ dDC subsets were analyzed by means of flow cytometry. The frequencies (percentages) of $EpCAM^+$ $CD103^-$ and $EpCAM^-$ $CD103^+$ cells among $CD11c^+$ MHC class II^+ DCs in the dermis are shown. *APC*, Allophycocyanin; *PE*, phycoerythrin.

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INVESTIGATIVE REPORT

In vitro Propagation and Dynamics of T cells from Skin Biopsies by Methods Using Interleukins-2 and -4 or Anti-CD3/CD28 Antibody-coated Microbeads

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In order to explore the mechanisms of inflammatory skin disorders, we established two methods of expanding skin-derived lymphocytes, one using high levels of interleukin (IL)-2 and IL-4 (method A) and the other using low levels of cytokines and anti-CD3/CD28 microbeads (method B). Both methods provide advantages for functional studies. With either of these two, we could obtain more than 10^7 cells/ from a 3 mm skin biopsy in 21 days from 23 out of 26 biopsies of various skin diseases. The relevance of these cells was confirmed by shifted T-cell receptor β chain variable region (TCR-Vβ) repertoire and antigen-dependent proliferation in antigen-driven skin disorders. The propagation of skin-resident lymphocytes, seen especially in method A, seems to be mediated by a functional defect of regulatory T cells residing in skin sequentially expanding under the conditions of our methods. ***Key words: skin-derived lymphocytes; T-cell receptor repertoire; regulatory T cells.***

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Skin has an exquisite immune system that protects against invasion by pathogens. Dysregulation of the skin immune system may influence chronic inflammatory skin diseases, including atopic dermatitis (AD) and psoriasis (1). An ideal way of exploring the mechanisms of such conditions would be to obtain large numbers of inflammatory cells resident in skin. However, it is difficult to expand a low number of cells from skin explants to obtain sufficient numbers of cells to investigate, and special techniques and devices are required (2, 3). We describe here the establishment of two simple methods of expanding skin-derived lymphocytes, which provide advantages for functional studies.

MATERIALS AND METHODS

Patients

Twenty-three patients with AD (6 men, 3 women; mean age 44 years (range 36–61 years)), psoriasis (6 men, 1 woman; mean age 47.5 years (range 26–70 years)), acute/chronic eczema, which could not be further defined (2 men, 2 women; mean age 64 years (range 55–87 years)), pityriasis lichenoides (1 man, age 68 years) or drug eruption (2 men, age 56 and 64 years) were included in the study. Two healthy individuals (age 48 and 64 years) donated normal skin and a subject with an erythematous lesion following a tuberculin skin reaction (1 man, 64 years) also participated in this study. A total of 26 skin specimens were obtained from these 25 patients/persons. All of the patients were informed about the purpose of this study and agreed to participate. All studies were conducted in accordance with the Declaration of Helsinki Principles for Human Tissue Research and were approved by the Institutional Review Board of Aarhus University and Hamamatsu University School of Medicine.

Reagents, monoclonal antibodies and culture media

Fluorescein isothiocyanate (FITC)-labelled anti-Vαβ common frame and anti-cutaneous lymphocyte antigens (CLA) monoclonal antibodies (MoAbs) were obtained from PharMingen, Sorrent Valley, CA, USA. Twenty-four T-cell receptor β chain variable region (TCR-Vβ) gene products were analysed with IOtest Beta Mark, TCR-Vβ repertoire kit (Beckman Coulter Co., Marseille, France). FITC- and phycoerythrin (PE)-labelled anti-CD3 (SK7), anti-CD4 (SK3), and anti-CD8 (SK1) were obtained from Beckton Dickinson, San Jose, CA, USA. MoAbs against chemokine receptors were obtained from R & D System Inc. Minneapolis, MN, USA.

To detect the expression of Foxp3, a PE anti-human Foxp3 staining set (e-Bioscience, San Diego, CA, USA) was used according to the manufacturer's protocol.

Cells were cultured in RPMI-1640 (Gibco, Paisley, UK) supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), 5×10^{-5} M 2-mercaptoethanol, 1% of a 100 × mixture of non-essential amino acids (Gibco), antibiotics and pooled human AB serum or 10% heat-inactivated foetal calf serum. For culture of these cells, the medium was supplemented with recombinant human interleukin 2 (rIL-2, Takeda Pharmaceutical Co., Tokyo, Japan and R & D System Inc.) and/or recombinant human IL-4 (R & D System Inc.).

Establishment of skin-derived lymphocyte cell lines

Skin-derived lymphocyte cell lines were established by two methods (A and B), as follows.

Method A. A 3-mm skin biopsy was cut into 1 mm square pieces with a sharp blade and immersed in 10 ml RPMI-1640 containing IL-2 (1,000 U/ml) and IL-4 (250 U/ml) in a 25 ml culture bottle (Corning) for 14–21 days. When the cell density yielded more than 10^6 cells/ml, half of the culture medium was exchanged with RPMI-1640, as above, containing IL-2 and IL-4 at the same concentrations.

Method B. A 3-mm skin biopsy was cut as in method A and cultured in 10 ml RPMI-1640 containing IL-2 (50 U/ml) and 10 µl anti-CD3/CD28 Ab-coated microbeads (CD3/CD28 T-cell expander, Dynal Biotech, Oslo, Norway) in a 25 ml culture bottle for 7 days. On day 8, the cells were harvested and the bound microbeads were detached with a magnetic device (Dynal), and transferred into a new flask containing RPMI-1640 with IL-2 and new microbeads at the same concentration (re-stimulation). At 6–8-day intervals re-stimulation was performed in order to further expand the cells for 2 months.

Cell preparations for antigen-presenting cells

Freshly isolated peripheral blood mononuclear cells (PBMC) were incubated in a 6-well-plate for 45 min, and the adherent cells were harvested and cultured in RPMI with serum containing GM-CSF (10 ng/ml, R & D) and IL-4 (10 ng/ml) for 14–21 days to obtain monocyte-derived dendritic cells as antigen-presenting cells (APCs).

Lymphocyte proliferation assay with 5- or 6-(N-succinimidyloxycarbonyl)-fluorescein 3',6'-diacetate labelling method

After labelling with 5- or 6-(N-succinimidyloxycarbonyl)-fluorescein 3',6'-diacetate (CFSE), as described previously (4), cells were cultured in 96-well flat-bottomed plates (Corning Glass Works, Corning, NY, USA) in the presence or absence of antigens (tetanus toxoid (TT) 3 fpu/ml, purified protein derivatives (PPD) 1–10 µg/ml or salazosulfapyridine10 µg/ml and APCs in selected cell lines, and were analysed with flow cytometry after 3 days culture.

Flow cytometric analysis

Aliquots of 10^5 cells were stained with FITC-, PE- and PerCP-conjugated MoAbs, and analysed as described previously (4).

Statistical analysis

Mann-Whitney *U* test was used for non-parametric analysis and Student's *t*-test for parametric analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Propagation of skin-derived lymphocytes from a skin biopsy

The cell number increased progressively to more than 10^7 cells/specimen from all the skin pieces in method A (*filled symbols*) and B (*open symbols*), in 23 out of 26 biopsies (Fig. 1a and b). In method A, the proliferation activity peaked between 14 and 20 days in culture and decreased over the next 30 days (Fig. 1b). The optimal proliferation response was observed at a cell density of approximately 5×10^5 cells/ml (Fig. 1b). In method B, although the proliferation activity was inferior to

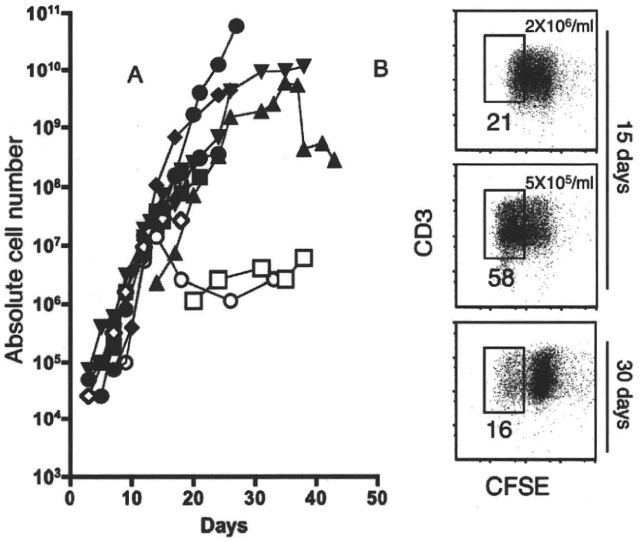

Fig. 1. Proliferation of cells derived from skin specimens. (a) Absolute number of cells expanded from skin lesions by methods A (*filled symbols*) and B (*open symbols*). Total cell number per skin piece was indicated. The x-axis indicates culture period. Circle, AD; square, drug eruption; triangle, psoriasis. (b) Cell division activity depending on cell density and culture days. After labelling with 5- or 6-(N-succinimidyloxycarbonyl)-fluorescein 3',6'-diacetate, cells harvested at day 15 and day 30 were cultured for 72 h at different cell densities (2×10^6/ml and 5×10^5/ml), followed by flow cytometry (FCM) analysis. Numbers indicate the percentage of divided cells in the total cells, as shown in a box.

that in method A (Fig. 1a), periodic stimulations with anti-CD3/CD28 Ab-coated microbeads could maintain cell viability for at least 3 months in 12 out of 14 biopsies.

Analysis of phenotype and TCR-Vβ usage of skin-derived cell lines

The phenotypes of cells expanded by methods A and B from skin lesions were examined for their TCR-Vβ repertoire of lymphocytes. In both methods, the expanded cells from all the specimens were $CD3^+CD14^-$

Table I. *Phenotype of expanded cells during culture*

			D12*	D20*	D28*
Method A	PPD reaction	CD4	77.5	14.0	n.d.
		CD8	20.3	85.9	n.d.
	Psoriasis	CD4	59.6	93.1	99.5
		CD8	24.6	4.7	0.5
	Drug eruption	CD4	81.1	49.0	15.9
		CD8	17.6	47.9	77.5
	Normal skin	CD4	97.6	88.0	25.5
		CD8	0.15	11.7	74.2
Method B	Drug eruption	CD4	35.6	29.8	11.4
		CD8	64.4	67.6	85.2
	Normal skin	CD4	60.5	55.5	40.0
		CD8	35.2	45.0	58.0

The percentage of cells positive for CD4/CD8 among total lymphocytes is indicated.
*Indicates culture days. n.d.: not done.
PPD: purified protein derivatives.

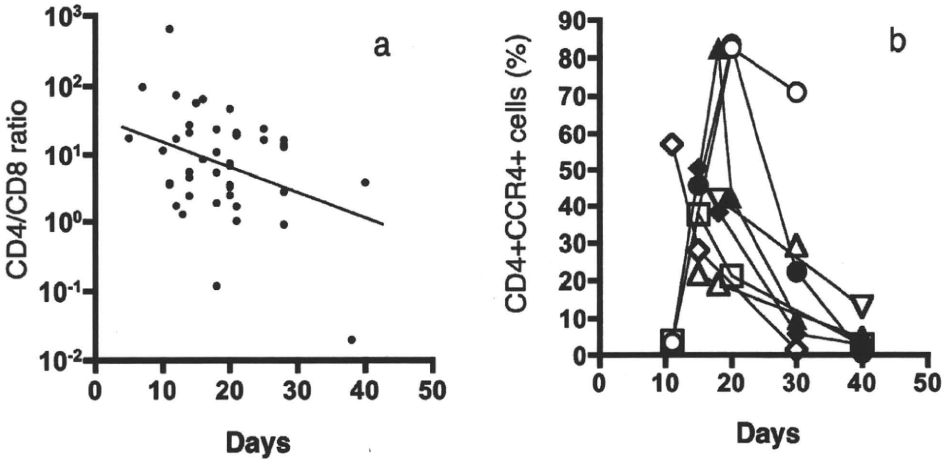

Fig. 2. Phenotypic changes of expanded cells during culture. (a) Ratio of $CD4^+$ cell number to $CD8^+$ cell number during culture. Each plot indicates the ratio at that time-point. (b) Percentage of $CD4^+CCR4^+$ cells in total cells expanded from atopic dermatitis ($n=4$, *open symbols*) and psoriasis ($n=4$, *filled symbols*) lesions during culture. Symbols indicate individual patients.

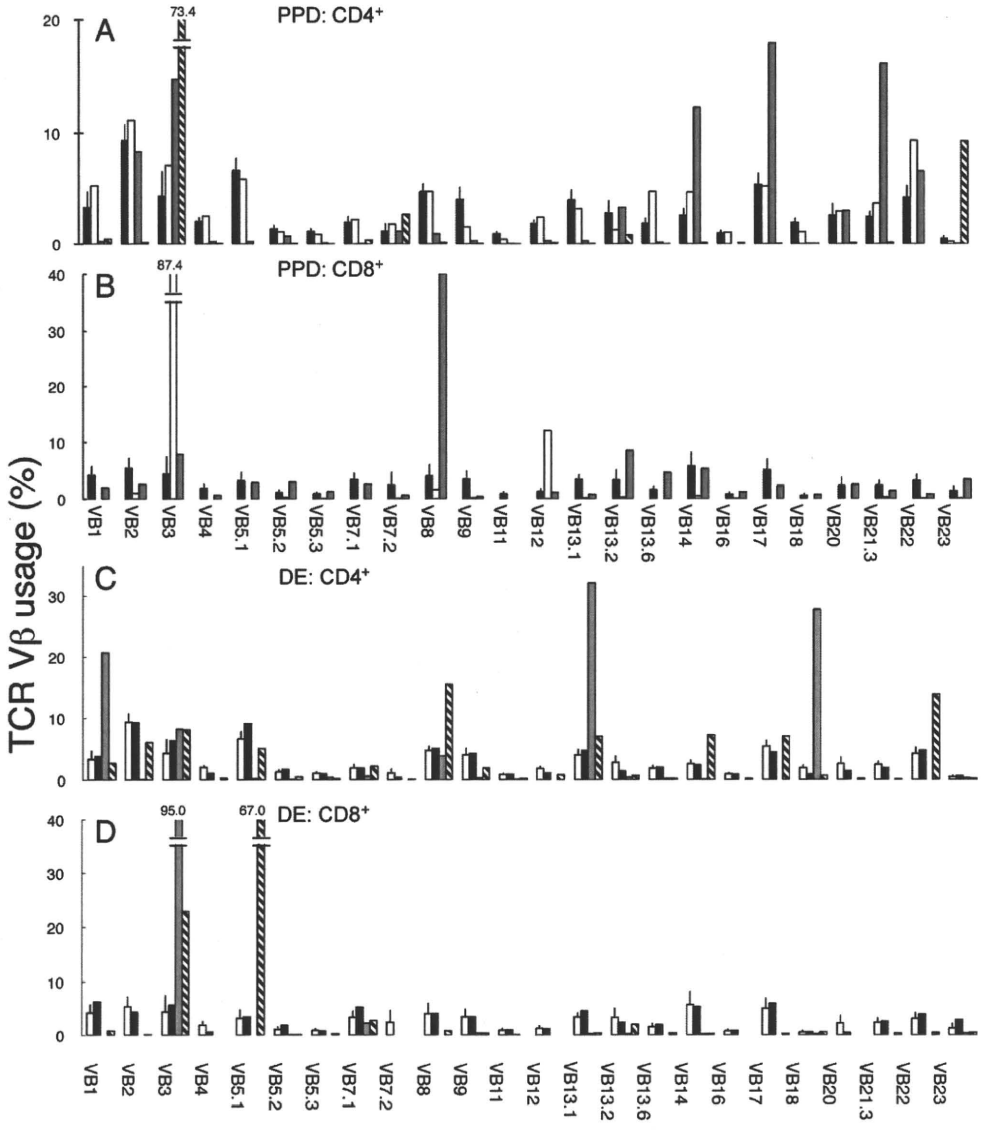

Fig. 3. T-cell receptor β chain variable region (TCR-Vβ) repertoire of T cells expanded from skin. (A and B) TCR-Vβ usage of cells expanded by method A from the positive tuberculin test (*grey*) and the normal site 5 cm away from the lesion (*hatched*) and circulating lymphocytes of normal subjects (*black*) and the tested subject (*white*) was analysed by FCM using antibodies against Vβs at the gate of (A) $CD4^+$ cells and (B) $CD8^+$ cells. (C and D) TCR-Vβ usage of cells expanded by method A (*dark-grey*) and method B (*light-grey*) from drug eruption (DE) lesions and circulating lymphocytes of normal subjects (*black*) and the patient (*white*) was analysed by FCM using antibodies against Vβs at the gate of (C) $CD4^+$ cells and (D) $CD8^+$ cells. *Vertical bar*: standard deviation. Numbers indicate the percentage of total cells.

$CD19^-CD20^-$ cells, indicating T lymphocytes. A decrease in CD4/CD8 ratio was noted (Table I and Fig. 2a) with a transient increase in $CD4^+CCR4^+$ cells (Fig. 2b) during the culture in method A, but not in method B.

We established two cell lines by method A from the skin samples of a positive tuberculin lesion and clinically normal skin 5 cm away from the tuberculin lesion in the same individual (Fig. 3A and B). $CD4^+$ cells from the tuberculin reaction showed preferential usage of Vβ3, Vβ14, Vβ17 and Vβ21.3, while those from normal skin predominantly expressed Vβ3 and Vβ23 (Fig. 3A). Among $CD8^+$ cells, the expanded cells from the lesion highly expressed Vβ8 (Fig. 3B), whereas all the cells from the normal skin site were negative for CD8. This suggests that $CD8^+$ T cells accumulating in inflamed skin expand easily compared with normal skin.

We next compared the Vβ usage of cells expanded from drug eruptions using methods A and B at 21 days (Fig. 3C and D). We observed divergence in the T-cell receptor repertoire of expanded cells between the two methods. While $CD4^+$ cells bearing Vβ1, Vβ13.1 and Vβ18 proliferated preferentially by method A, those positive for Vβ8, Vβ14 and Vβ22 expanded significantly by method B (Fig. 3C). Additionally, we found that 95% of $CD8^+$ cells were positive for Vβ3 in method A, and 65% were Vβ5.1 in method B (Fig. 3D). The highly skewed TCR-Vβ repertoire of the skin-derived cells and their remarkable difference from the circulating lymphocytes suggest special compartmentalization of skin-specific T cells.

Relevance of lymphocytes derived from inflammatory skin lesions

To investigate whether the lymphocytes expanded from skin lesions by these two methods were pathogenic, we investigated the immune responses of expanded lymphocytes to causative antigens in the tuberculin reaction and drug eruption. Despite an absence of proliferative response in the culture without PPD or APCs, irrespective of their CD4 or CD8 expression, CFSE-labelled cells expanded from the tuberculin reaction by method A were proliferating in response to PPD antigens, but not to tetanus toxoid (TT) in the presence of autologous APCs (Fig 4a). The cells expanded from normal skin in the tested individual did not respond to PPD. In the same way, we found drug-specific proliferation of skin-resident cells from salazosulfapyridine-induced eruption lesion by method B (Fig 4b), although $CD4^+$ cells predominantly expanded rather than $CD8^+$ cells (data not shown). These observations indicated that the cells expanded from skin lesions by our methods contained antigen-specific T cells in the antigen-driven skin diseases.

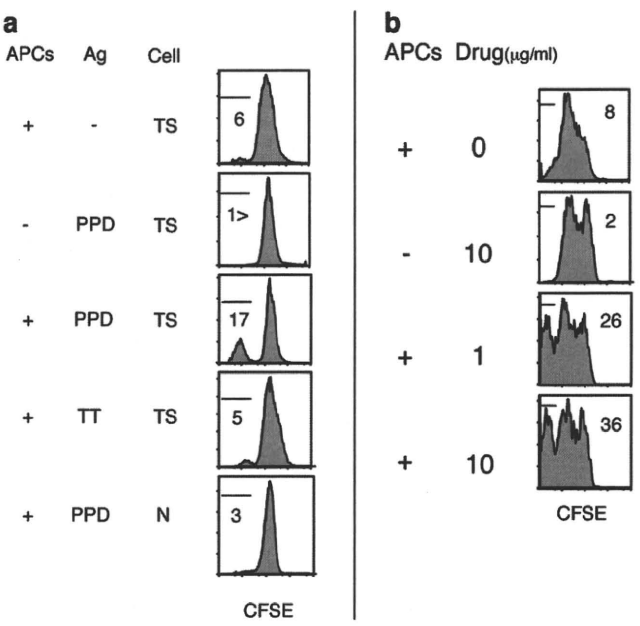

Fig. 4. Antigen response of the expanded cells by (a) method A and (b) method B in inflammatory conditions. After labelling with 5-or 6-(N-succinimidyloxycarbonyl)-fluorescein 3',6'-diacetate (CFSE), the expanded cells were cultured for 72 h with or without antigen-presenting cells (APCs) and in the presence of antigens, followed by FCM analysis. (a) Proliferation of the cells expanded by method A from positive tuberculin test site (TS) in response to PPD antigens. +: added; –: not added; TT: tetanus toxioid; N: cells expanded from normal site near the positive test site in the same individual. Number indicates percentage of total cells. (b) Proliferation of the cells expanded by method B from the drug eruption lesion in response to the culprit drug, salazosulfapyridine (drug, 0, 1 and 10 μg/ml). +: added; –: not added. Numbers indicate percentage of total cells.

Transient expansion of $CD4^+CD25^+Foxp3^+$ cells by high level of IL-2 and anti-CD3/CD28 microbeads

A high level of IL-2 promotes vigorous expansion of natural regulatory T cells (Tregs), being parallel to augmentation of an anti-tumour effect in patients with cancer (5, 6). Because of the high IL-2 level in method A, we investigated the number of Tregs among $CD4^+$ cells expanded from various disease skins including AD ($n=5$) and psoriasis ($n=4$) by enumeration of cells co-expressing CD25 and Foxp3 (6). In method A, $CD4^+CD25^+Foxp3^+$ cell population was $5.0\% \pm 4.7\%$ (mean ± SD) at day 10–13 (Fig 5A–C) and significantly increased to $23.2\% \pm 17.4\%$ from day 14 to day 20, while they decreased to $7.9\% \pm 6.7\%$ after day 21 and disappeared after day 35. In cultures from AD lesions, comparable increments of $CD4^+CD25^+Foxp3^+$ cells were found between methods A (40.1%) and B (32%) at day 16.

Cytotoxic activity of blood lymphocytes against expanded skin-resident cells

We investigated the cytotoxic activity of blood lymphocytes against expanded cells from skin lesions of AD

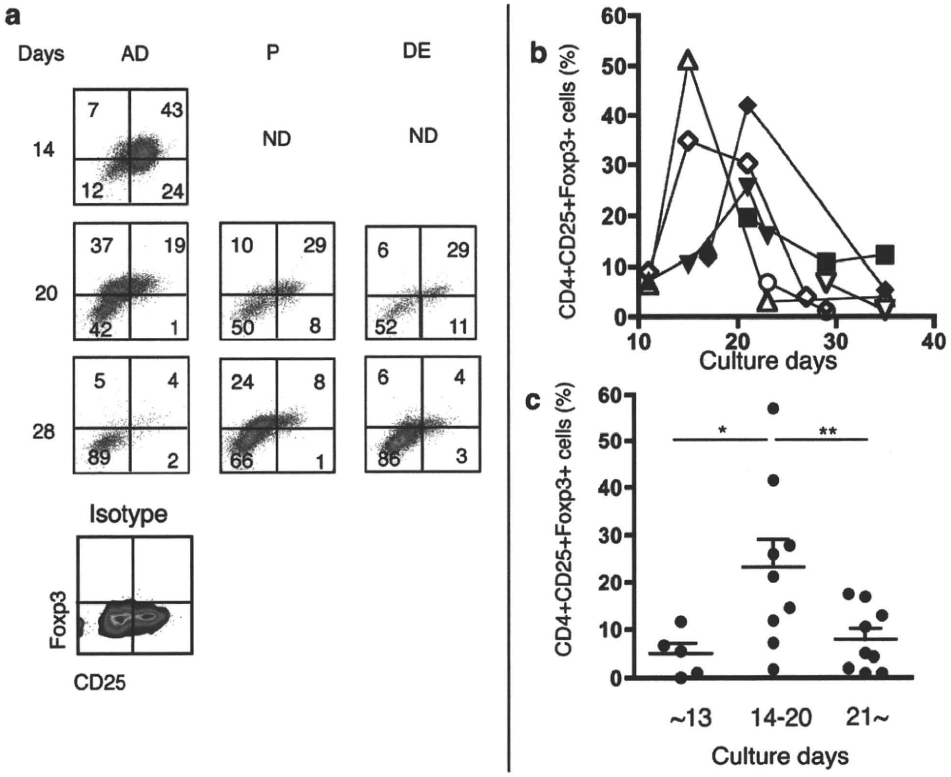

Fig. 5. $CD4^+CD25^+Foxp3^+$ cells during cultures. (a) Percentage of $CD4^+CD25^+Foxp3^+$ cells in cells expanded by method A from skin lesions of atopic dermatitis (AD), psoriasis (P) and drug eruption (DE) during culture. After reaction with fluorescence-conjugated Abs against CD4, CD25 and Foxp3, cells were analysed with FCM at the gate of $CD4^+$ lymphocyte population. Numbers indicate gated population in the total cells. Representative results shown. (b) Percentage of $CD4^+CD25^+Foxp3^+$ cells in cells expanded from atopic dermatitis ($n=5$, *open symbols*) and psoriasis ($n=4$, *filled symbols*) lesions by method A during culture. (c) Percentage of $CD4^+CD25^+Foxp3^+$ cells in cells expanded by method A during culture. Plots include all data for various skin diseases ($n=10$) in this study (some values are missing). $*p<0.02$, $**p<0.03$. Mann-Whitney U test. ND: not done.

($n=6$), psoriasis ($n=4$) and a positive tuberculin test. However, no cytotoxic activity was detected.

DISCUSSION

In order to obtain a large number of skin-resident T cells, we established two methods of expanding T-cell lines from 23 out of 26 skin pieces, including AD, drug eruption, psoriasis, acute/chronic dermatitis, pityriasis lichenoides, PPD reaction and normal skin. Our methods rendered more than 10^7 cells for 21 days, and are superior to the previous "gold-standard" method of Cavani et al. (7) and the recently-developed method that can propagate at most 2.5×10^5 cells from a skin sample for 21 days (8).

Although large numbers of skin-resident T cells could be used to obtain a direct insight into the mechanisms central to the skin disease in question, it is practically difficult to obtain high numbers of such cells. Only low numbers of skin-resident cells are obtained using pre-existing methods of mechanical dissociation combined with chemical reagents (2, 8), and the cells need to be expanded in culture in order to obtain larger numbers to analyse the cell functions, as in the case of establishing T-cell clones and lines with complicated culture techniques (8). To overcome these issues, researchers have developed various methods, including cell culture with a high-dose of IL-2 alone (9), plus IL-4 (10–13) and special devices (3, 7). However, these methods cannot ensure that the expanded cells are relevant to pathogenic cells in the skin. The present study clearly showed the functional relevance of expanding skin cells.

A difference of our methods from those of others is that the skin specimens are placed floating in the medium in a 25 ml flask. Intact skin explants may supply skin tissue-releasing factors that are important for survival and proliferation of skin-resident lymphocytes such as IL-7 (7). Considering that maintaining the viability of cultured lymphocytes usually requires cell-cell contact, the cell density in our methods may be low, since lymphocytes emigrated from skin pieces are less than 100 cells in 10 ml culture media on the first day, suggesting that high cell density is not important to expand the skin-resident cells using our methods. One explanation may be that our culture conditions prevent skin-resident lymphocytes from the inhibitory effects of skin-resident Tregs.

We observed a phenotypic divergence from CD4-dominance to CD8-dominance among the expanding cells between day 18 and 28 in method A, confirming a previous report (12), with a transient increase in $CD4^+CCR4^+CD25^+Foxp3^+$ Tregs preceding this phenomenon. It has been shown that high IL-2 treatment propagates natural Tregs, but that they lose their function simultaneously, being paralleled with augmentation of cytotoxicity against cancer cells (5, 14). Alternatively, the high level of IL-2 in method A may promote early incrementation of $CD4^+$ cells that predominantly express high-affinity IL-2 receptors and, subsequently, an expansion of $CD8^+$ cells that predominantly express low-affinity IL-2 receptors, which might depend on

their profile of IL-2 receptor expression (15). Although method A is a very simple way to obtain a large number of skin-resident cells, including Tregs, it must be noted that these cells may be phenotypically and functionally biased under Th2 cytokine condition. On the other hand, method B is useful for functional investigation of skin-resident lymphocytes, although repeated stimulations with anti-CD3/CD28 microbeads are necessary for propagation.

Easy isolation and expansion of skin-resident cells will enable further investigation of how T cells contribute to cutaneous pathology in skin diseases; the two methods described here will be a beneficial strategy for use in investigating this issue.

ACKNOWLEDGEMENT

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The authors declare no conflict of interest.

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Eur J Dermatol 2010; 20 (1): 1-2

CORRESPONDENCE

Contact immunotherapy-induced Renbök phenomenon in a patient with alopecia areata and psoriasis vulgaris

Happle *et al.* first introduced the term "Renbök phenomenon", derived from the reversal of "Köbner", to describe the observation of normal hair growth in psoriatic lesions in patients with co-existing psoriasis and alopecia areata (AA) [1]. Although the exact mechanism of this unique phenomenon is unknown, it has been proposed that biological events inherent in psoriasis may act on hair follicles to restore hair growth in AA. We herein describe the Renbök phenomenon related to contact immunotherapy in a patient suffering from AA and psoriasis vulgaris.

A 15-year-old girl presented with a 3-year history of patchy hair loss on the vertex of the scalp with a gradual increase in the number of lesions. In addition, scaly and erythematous eruptions appeared on her scalp and upper extremities one year after the onset of scalp hair loss. Initial examination at our outpatient clinic revealed multiple hair loss patches on her scalp and scaly lesions on her scalp and upper extremities. Interestingly, the presence of terminal hair coincided with the scaly lesions *(figure 1A)*. There was no associated systemic disease nor was there any family history of AA or psoriasis vulgaris.

Hematoxylin and eosin (H-E)-stained sections of a biopsy specimen from a hair loss patch revealed lymphocyte infiltration around atrophic hair follicles, compatible with the diagnosis of AA. The biopsy specimen from a scaly lesion of the right antebrachium showed parakeratosis, psoriasiform acanthosis, thin but club-shaped rete ridges, Munro's microabscesses and perivascular infiltration of lymphocytes. Results of laboratory studies including a hemogram and blood chemistry tests and thyroid autoantibodies were normal or negative. She had HLA-DQB1* 0301 (a gene susceptibility to AA) but was negative for HLA-Cw6 and HLA-DR7, which are genetic loci relating to psoriasis [2].

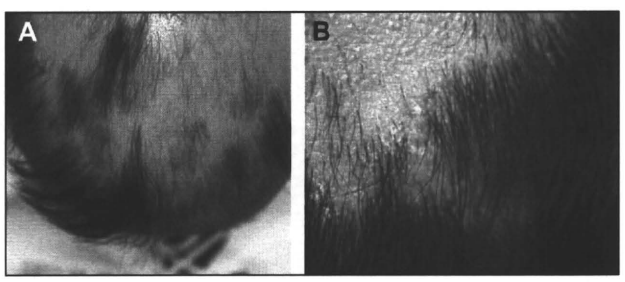

Figure 1. A) Extensive hair loss was observed on the vertex and occipital region of the head. **B)** Concomitant hair regrowth and psoriasis on the scalp 1 month after contact immunotherapy.

Contact immunotherapy with squaric acid dibutylester (SADBE), and topical maxacalcitol and betamethasone butyrate propionate were instituted for AA and psoriasis, respectively. Three months following contact immunotherapy, terminal hairs re-grew but psoriatic lesions developed at the sites of SADBE application *(figure 1B)*, which were then successfully treated with maxacalcitol lotion. Other pre-existing psoriatic lesions responded well with the 4 weeks of the topical treatment. One year later, the hair loss and psoriasis were markedly improved, although a few hair loss patches occasionally recurred.

In our case, contact immunotherapy was effective for AA but also induced psoriatic lesions at the site of treatment. Orecchia *et al.* report a case of alopecia universalis in which there was a concomitant appearance of hair regrowth and psoriatic plaques in the same area following contact immunotherapy with SADBE [3]. Contact immunotherapy appears to modulate cytokine production in the skin with a decrease in the mRNA expression of interferon (IFN)-γ while mRNA for IL-2, IL-8, IL-10 and tumor necrosis factor (TNF)-α is increased [4]. AA is regarded as a tissue-specific autoimmune disease against melanin-associated proteins in the hair follicle [5]. IFN-γ may contribute to initiating the disease process by collapsing the hair follicle immune privilege and resulting in the exposure of autoantigens [5]. On the other hand, TNF-α is a crucial cytokine in psoriatic lesions [6]. Therefore, we propose that a change in the cytokine milieu due to contact immunotherapy may play an important role in both the improvement of alopecia and the induction of psoriasis. ■

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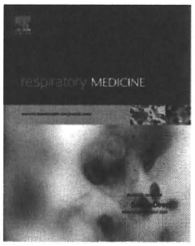

Distinct prognosis of idiopathic nonspecific interstitial pneumonia (NSIP) fulfilling criteria for undifferentiated connective tissue disease (UCTD)

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Summary
Background: Although idiopathic nonspecific interstitial pneumonia (NSIP) was initially identified as a provisional diagnosis, the 2008 American Thoracic Society Project concluded that idiopathic NSIP is a distinct form of idiopathic interstitial pneumonia. However, an association between idiopathic NSIP and autoimmune diseases still attracts interest. In this context, a recent study proposed an intriguing concept that idiopathic NSIP is the pulmonary manifestation of undifferentiated connective tissue disease (UCTD). However, this has not been confirmed in a large number of patients with idiopathic NSIP. The present study was conducted to investigate the proportion and characteristics of patients with idiopathic NSIP who meet the criteria for UCTD.
Methods: We reviewed 47 consecutive patients with idiopathic NSIP and examined whether they met prespecified criteria for UCTD. Furthermore, we compared the clinical

Abbreviations: NSIP, nonspecific interstitial pneumonia; UIP, usual interstitial pneumonia; DIP, desquamative interstitial pneumonia; IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; UCTD, undifferentiated connective tissue disease; CTD, connective tissue disease; PM, polymyositis; DM, dermatomyositis; SS, primary Sjögren syndrome; RA, rheumatoid arthritis; SLE, systemic lupus; SSc, systemic sclerosis; BAL, bronchoalveolar lavage.

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characteristics between patients fulfilling the UCTD criteria (UCTD-NSIP) and those not meeting them (Non-UCTD-NSIP).
Results: Of 47 patients with idiopathic NSIP, 22 (47%) met the UCTD criteria. Common symptoms associated with connective tissue diseases (CTDs) were skin change (50%) and Raynaud's phenomenon (41%) in UCTD-NSIP. UCTD-NSIP showed a female predominance and significantly higher percentages of lymphocytes with a lower CD4/CD8 ratio in bronchoalveolar lavage than Non-UCTD-NSIP. Interestingly, UCTD-NSIP had a significantly better survival than Non-UCTD-NSIP.
Conclusions: Idiopathic NSIP included subjects who fulfilled the UCTD criteria, and these subjects had different clinical characteristics with significantly better outcome than those who did not meet the criteria. These data suggest that a part, but not all, of patients with idiopathic NSIP show CTD-like features with a distinct prognosis.

Introduction

Nonspecific interstitial pneumonia was originally described as a pathologic pattern distinct from other defined interstitial pneumonias, such as usual interstitial pneumonia (UIP) and desquamative interstitial pneumonia (DIP), by Katzenstein and Fiorelli.[1] NSIP has also been shown to be associated with a variety of conditions, including connective tissue diseases (CTDs), drug reactions, and organic dust exposures.[1–6] Thus, the 2002 Joint Statement of American Thoracic Society (ATS) and European Respiratory Society (ERS) on the classification of idiopathic interstitial pneumonias (IIPs) described idiopathic NSIP as a provisional diagnosis to be further defined.[7] Interestingly, recent studies have demonstrated that NSIP is the most common histologic pattern in CTD-associated interstitial pneumonias,[2–4,8,9] and that patients with idiopathic NSIP often exhibit CTD-like features, such as autoantibodies.[10,11] The precise relationship between idiopathic NSIP and CTDs remains to be further clarified.

Kinder et al. recently proposed a very interesting hypothesis: idiopathic NSIP is the pulmonary manifestation of undifferentiated connective tissue disease (UCTD).[12] UCTD is characterized by the presence of signs and symptoms suggestive of a systemic autoimmune disease but they do not meet the criteria for defined CTDs, such as systemic lupus erythematosus (SLE), Sjögren syndrome (SS), and rheumatoid arthritis (RA).[13–17]

However, no validated criteria for the diagnosis of UCTD have been established so far. Kinder et al. proposed prespecified criteria for UCTD and investigated the proportion and characteristics of patients fulfilling their UCTD criteria in IIPs.[12] They showed that IIP patients who met the UCTD criteria had distinct features, including a female predominance, high incidence of ground-glass opacity on high-resolution computed tomography (HRCT), and NSIP pattern on surgical lung biopsy. Remarkably, the majority of patients with idiopathic NSIP (88%) met the UCTD criteria, while only a small proportion (5%) of those with idiopathic pulmonary fibrosis (IPF) fulfilled the criteria. Thus, Kinder et al. concluded that idiopathic NSIP appears to be an autoimmune disease, the pulmonary manifestation of UCTD. This hypothesis is attractive but needs to be confirmed. In particular, the study of Kinder et al. included only a small number of patients with idiopathic NSIP (17 cases). In contrast to Kinder's study, the ATS project recently supported the notion that idiopathic NSIP is a distinct clinical entity of IIPs.[18] Out of the original 306 cases of idiopathic NSIP, however, only 67 cases were identified as definite or probable idiopathic NSIP by a dynamic integrated multidisciplinary approach in the ATS project. This project also mentioned several future issues to be investigated for further confirmation of the clinical entity of idiopathic NSIP, one of which is to determine whether idiopathic NSIP is a manifestation of an autoimmune disease.

The present study was conducted to investigate the proportion of patients with idiopathic NSIP fulfilling the UCTD criteria proposed by Kinder et al. in a larger population, and to define the clinical characteristics of those patients. Furthermore, we attempted to clarify the significance of UCTD diagnosis in idiopathic NSIP.

Patients and methods

Patients and diagnostic criteria

We studied 62 consecutive patients with idiopathic NSIP who underwent open or thoracoscopic lung biopsy at our facilities from 1990 to 2009. The diagnosis of idiopathic NSIP was based on history, physical examination, HRCT, and histologic examination, in accordance with the ATS/ERS consensus classification.[7] At the initial diagnosis, none of the patients fulfilled the American College of Rheumatology (ACR) criteria for defined CTDs, such as RA, SS, systemic sclerosis (SSc), polymyositis/dermatomyositis (PM/DM), SLE, or mixed connective tissue disease (MCTD). The study protocol was approved by the Ethical Committee of the Hamamatsu University School of Medicine.

We used the criteria for UCTD proposed by Kinder et al. as defined in Table 1. Patients were diagnosed as having UCTD if they had at least one of symptoms associated with CTDs and at least one evidence of systemic inflammation listed in Table 1. Because there was the possibility of false-negative diagnosis of patients for whom fewer items listed as evidence of systemic inflammation in Table 1 were measured, the study subjects included patients with at least four items assessed as evidence of systemic inflammation.

Table 1 Diagnostic criteria for undifferentiated connective tissue disease.

Diagnostic criteria	Presence of
Symptoms associated with connective tissue disease	At least one of the following symptoms: 1. Raynaud's phenomenon 2. Arthralgias/multiple joint swelling 3. Photosensitivity 4. Unintentional weight loss 5. Morning stiffness 6. Dry mouth or dry eyes (sicca features) 7. Dysphagia 8. Recurrent unexplained fever 9. Gastroesophageal reflux 10. Skin changes (rash) 11. Oral ulceration 12. Nonandrogenic alopecia 13. Proximal muscle weakness
Evidence of systemic inflammation in the absence of infection	Positive findings for at least one of the following: 1. Antinuclear antigen 2. Rheumatoid factor 3. Anti-SCL 70 antibody 4. SS-A or SS-B antibody 5. Jo-1 antibody 6. Sedimentation rate (>two times normal), C-reactive protein

* Criteria are derived from Reference 12.

Data collection

Clinical data, including sex, age, smoking history, symptoms, treatment and outcome were obtained from patient medical records. Laboratory findings, pulmonary function tests, and bronchoalveolar lavage (BAL) data at the time of surgical lung biopsy were also recorded.

Pathological review

Lung biopsy specimens were independently reviewed by three pathologists (T.V.C., Y.N., S.I.) who were unaware of the clinical or physiological findings. In 8 cases, initial histological classification differed between the pathologist, but a consensus opinion on the overall histopathological pattern was reached. Histological classification was based on the previously published criteria for IIPs from the ATS/ERS.[7] In addition, the degree of each pathologic finding was semiquantitatively scored (absent 0, mild 1, moderate 2, and marked 3) by two pathologists (Y.N., S.I.). The pathologic findings scored included the following: alveolar inflammation, intra-alveolar macrophages, organizing pneumonia, germinal centers, fibrosis, fibroblastic foci, honeycombing, and pleural changes.

High-resolution computed tomography (HRCT)

HRCT examination of the lungs was performed on 1.0- or 1.5-mm-thick sections to evaluate radiographic abnormalities. The HRCT images were reviewed for the presence and distribution of each of the following sign: ground-glass attenuation, airspace consolidation, interlobular septal thickening, intralobular reticular opacity, thickening of bronchovascular bundles, traction bronchiectasis, honeycombing, and cysts.

Statistical analysis

For two-group comparisons involving binary data, we used the chi-square test. Comparisons involving continuous data were made using Mann–Whitney *U* test. The interobserver correlation was analyzed using Pearson's correlation coefficient. Cumulative survival probabilities were estimated using the Kaplan–Meier method. The log-rank test was used to compare survival among the groups of patients. Statistical analyses were performed using JMP Start Stastitics (SAS Institute Inc., NC, USA). A p value <0.05 was considered significant.

Results

Patient characteristics

Of the original 62 NSIP patients, six patients were excluded, because they developed PM/DM during the observation period following initial diagnosis. These six patients had fulfilled the UCTD criteria at the initial NSIP diagnosis. Among the remaining 56 patients, 47 had adequate data with $\geq$ four items as evidence of systemic inflammation among the diagnostic criteria for UCTD. Of these 47 patients, 22 (47%) met the criteria for UCTD proposed by Kinder et al.

Clinical characteristics of patients who met the criteria for UCTD (UCTD-NSIP) and those who did not meet them (Non-UCTD-NSIP) are shown in Table 2. The median age for the patients of UCTD-NSIP was similar to that for those of Non-UCTD-NSIP. The proportion of males and current smokers tended to be lower in UCTD-NSIP than in Non-UCTD-NSIP, but the differences were not statistically significant. Respiratory symptoms or signs did not significantly differ between them. In UCTD-NSIP, the most common symptom associated with CTDs was skin change (50%), followed by Raynaud's phenomenon (41%) and arthralgias/joint swelling (23%). Fourteen patients with UCTD-NSIP (64%) had $\geq$ two symptoms. In Non-UCTD-NSIP, the symptoms associated with CTDs were rarely observed.

Laboratory findings

No significant difference was found in the serum levels of lactate dehydrogenase (LDH), C-reactive protein (CRP), KL-6, or surfactant protein-D (SP-D) between UCTD-NSIP and Non-UCTD-NSIP (Table 3). There were trends for creatine phosphokinase (CPK) and sedimentation rate to be higher in

UCTD-NSIP than in Non-UCTD-NSIP, but the differences did not reach statistical significance.

Among autoantibodies, anti-nuclear antibody was most frequently found in UCTD-NSIP (68%) (Table 3). The incidence of positive anti-nuclear antibody was significantly higher in UCTD-NSIP than in Non-UCTD-NSIP ($p = 0.0262$), but the titers were not significantly different (median [range], 120 [40–320] and 120 [40–1280], respectively). The positive rates of rheumatoid factor of UCTD-NSIP were similar to those of Non-UCTD-NSIP. Anti-Jo1 antibody and PR3-ANCA were present exclusively in UCTD-NSIP.

No significant difference was found in the results of pulmonary function tests between UCTD-NSIP and Non-UCTD-NSIP, although FVC and diffusion capacity for carbon monoxide (DLco) tended to be lower in UCTD-NSIP than in Non-UCTD-NSIP (Table 3).

BAL was performed in 17 and 23 patients with UCTD-NSIP and Non-UCTD-NSIP, respectively. The percentage of BAL lymphocytes was significantly higher in UCTD-NSIP than in Non-UCTD-NSIP ($p = 0.0424$) (Table 3). In addition, the percentage of BAL macrophages and the CD4/CD8 ratio of BAL lymphocytes were significantly lower in UCTD-NSIP than in Non-UCTD-NSIP ($p = 0.0328$ and $p = 0.0145$, respectively).

Pathological findings

Cellular NSIP was histologically diagnosed in 2 of 22 patients with UCTD-NSIP and 3 of 25 those with Non-UCTD-NSIP (9.1% vs. 12.0%, respectively), and the remaining patients had fibrotic NSIP (Table 4). Regarding each pathological finding listed in Table 4, there was no significant difference in its score, although the scores of germinal center tended to be higher in UCTD-NSIP than in Non-UCTD-NSIP ($p = 0.0914$). Interobserver correlation in the score of each finding was statistically significant, but the r-values were not high (0.481–0.667).

Radiologic findings

As shown in Table 4, ground-glass attenuation and traction bronchiectasis were generally (>90%) seen in both UCTD-NSIP and Non-UCTD-NSIP. Airspace consolidation and thickening of bronchovascular bundles were more common in UCTD-NSIP than in Non-UCTD-NSIP ($p = 0.0534$ and $p = 0.0586$, respectively). Regarding distributions of abnormalities, lower zone predominance was prominent in both UCTD-NSIP and Non-UCTD-NSIP.

Table 2 Clinical characteristics of NSIP patients who fulfill the criteria for undifferentiated connective tissue disease compared with those who do not.

Characteristics	UCTD-NSIP patients[a] ($n = 22$)	Non-UCTD-NSIP patients[b] ($n = 25$)	*P* value
Age, yr	57 (24–77)[c]	58 (38–83)	n.s.
Gender, male/female	8/14	14/11	n.s.
Smoking habit, *n*			
Current/former/never	3/7/12	10/6/9	n.s.
Observation period, yr	3.8 (0.6–17.2)	4.1 (0.6–13.8)	n.s.
Symptoms and signs, *n* (%)			
Respiratory			
Cough	16 (73)	15 (60)	n.s.
Dyspnea	4 (18)	5 (20)	n.s.
Fine crackles	17 (77)	19 (76)	n.s.
Clubbing	2 (9)	2 (8)	n.s.
Systemic			
Skin change (rash)	11 (50)	1 (4)	< 0.0001
Raynaud's phenomenon	9 (41)	0 (0)	< 0.0001
Arthralgias/joint swelling	5 (23)	1 (4)	0.0477
Dysphagia	4 (18)	0 (0)	0.0258
Morning stiffness	4 (18)	0 (0)	0.0258
Proximal muscle weakness	4 (18)	0 (0)	0.0258
Sicca symptoms	3 (14)	1 (4)	n.s.
Recurrent fever	3 (14)	0 (0)	0.0287
Unintentional weight loss	1 (5)	0 (0)	n.s.
Photosensitivity	0 (0)	0 (0)	n.s.
GERD	0 (0)	1 (4)	n.s.
Oral ulceration	0 (0)	0 (0)	n.s.
Alopecia (nonandrogenic)	0 (0)	0 (0)	n.s.

UCTD, undifferentiated connective tissue disease: GERD, gastroesophageal reflux disease; n.s., not significant.
[a] NSIP patients who fulfill the criteria for UCTD.
[b] NSIP patients who do not fulfill the criteria for UCTD.
[c] Median (Range).

Treatment and outcome

Most of the patients were treated with corticosteroid or corticosteroid plus immunosuppressive agents (UCTD-NSIP 15 patients [77%]; Non-UCTD-NSIP 20 patients [80%]) (Table 5). Among the immunosuppressive agents, cyclosporine was most commonly given to the both groups. There was no significant difference in the percentage of patients receiving immunosuppressive agents or duration of the therapy between the two groups. Only one patient (5%) with UCTD-NSIP died of respiratory failure during the observation period, while eight patients (32%) with Non-UCTD-NSIP patients died. The difference was statistically significant ($p = 0.0170$). No patients with cellular NSIP died during the observation period in UCTD-NSIP or Non-UCTD-NSIP.

Table 3 Laboratory and bronchoalveolar lavage findings of NSIP patients who fulfill the criteria for undifferentiated connective tissue disease compared with those who do not.

Characteristics	UCTD-NSIP[a]	Non-UCTD-NSIP[b]	*P* value
LDH, IU/L	333 ± 149[c]	293 ± 104	n.s.
CPK, IU/L	252 ± 415	94 ± 50	n.s.
CRP, mg/dL	0.87 ± 1.00	0.60 ± 1.05	n.s.
Sedimentation rate, mm/hr	38 ± 7	22 ± 16	n.s.
KL-6, U/mL	1690 ± 1194	1792 ± 1410	n.s.
SP-D, ng/mL	261 ± 203	263 ± 132	n.s.
Anti-nuclear antibody	15/22 (68)[d]	9/25 (36)	0.0262
Rhematoid factor	3/21 (14)	4/25 (16)	n.s.
Anti-SCL 70 antibody	0/19 (0)	0/15 (0)	n.s.
Anti-SSA antibody	3/21 (14)	1/16 (6)	n.s.
Anti-SSB antibody	0/21 (0)	0/16 (0)	n.s.
Anti-Jo1 antibody	2/19 (11)	0/16 (0)	n.s.
Anti-centromere antibody	0/16 (0)	0/6 (0)	n.s.
Anti-RNP antibody	1/14 (7)	0/12 (0)	n.s.
Anti-double strand DNA antibody	1/18 (6)	0/17 (0)	n.s.
Anti-Sm antibody	0/10 (0)	0/9 (0)	n.s.
MPO-ANCA	0/12 (0)	0/13 (0)	n.s.
PR3-ANCA	1/12 (8)	0/14 (0)	n.s.
PaO_2 on room air, Torr	78 ± 13	78 ± 14	n.s.
FVC, % predicted	61 ± 15	70 ± 22	n.s.
DLco, % predicted	60 ± 17	72 ± 28	n.s.
Bronchoalveolar lavage (BAL)	$n = 17$	$n = 23$	
Total cell count, x 10^5/mL	2.62 ± 1.86	2.32 ± 2.07	n.s.
Cellular profile, %			
Macrophages	61.6 ± 30.0	84.1 ± 9.7	0.0328
Lymphocytes	28.9 ± 29.1	11.4 ± 9.8	0.0424
Neutrophils	5.6 ± 8.2	2.7 ± 2.7	n.s.
Eosinophils	3.2 ± 4.0	2.1 ± 2.8	n.s.
CD4/CD8 ratio of BAL lymphocytes	1.03 ± 1.20	2.49 ± 2.83	0.0145

UCTD, undifferentiated connective tissue disease; LDH, lactate dehydrogenase; SP-D, surfactant protein D; MPO-ANCA, myeloperoxidase antineutrophil cytoplasmic autoantibody; PR3-ANCA, proteinase 3-antineutrophil cytoplasmic antibody; VC, vital capacity; FEV1, forced vital capacity in 1 s; DLco, diffusion capacity for carbon monoxide; BAL, bronchoalveolar lavage; n.s., not significant.

[a] NSIP patients who fulfill the criteria for UCTD.

[b] NSIP patients who do not fulfill the criteria for UCTD.

[c] Mean ± SD.

[d] The number of positive results/the number tested (%).

Survival

A comparison of survival curves between the two groups is shown in Fig. 1. Patients with UCTD-NSIP had a significantly better survival rate than those with Non-UCTD-NSIP (5-year survival, 100% vs. 58%, respectively; $p = 0.0092$).

Discussion

The present study demonstrated that about half of patients with idiopathic NSIP met the criteria for UCTD proposed by Kinder et al. Comparing NSIP patients fulfilling the criteria for UCTD (UCTD-NSIP) with those who did not meet the criteria (Non-UCTD-NSIP), patients with UCTD-NSIP had a significantly higher percentage of BAL lymphocytes with a lower CD4/CD8 ratio. Interestingly, patients with UCTD-NSIP had a significantly better survival than those with Non-UCTD-NSIP. These data suggest that UCTD diagnosis based on the criteria of Kinder et al. is associated with favorable prognosis in idiopathic NSIP.

Between patients with UCTD-NSIP and those with Non–UCTD-NSIP, besides the BAL findings and prognosis, several differences were noted. A female predominance (64%) was found in UCTD-NSIP, but not in Non-UCTD-NSIP. A large proportion of patients with UCTD-NSIP presented with two or more CTD-associated symptoms and/or signs, while those with Non-UCTD-NSIP scarcely had them. On HRCT, airspace consolidation and thickening of bronchovascular bundles tended to be found more frequently in UCTD-NSIP than in Non-UCTD-NSIP (68.2% vs. 40.0%, $p = 0.0534$; 63.6% vs. 36.0, $p = 0.0586$, respectively) Taken together, these data suggest that patients fulfilling the UCTD criteria of Kinder et al. may have distinct characteristics in idiopathic NSIP.

We confirmed the study of Kinder et al. showing that idiopathic NSIP included subjects fulfilling their UCTD criteria. However, there were several discrepancies between this previous work and our study. First, the proportion of patients who met the criteria of Kinder et al. was lower in our NSIP patients than in this previous work (47% vs. 88%, respectively). Kinder et al. proposed the interesting notion that the clinical entity of idiopathic NSIP is the lung manifestation of UCTD, because most of their patients with idiopathic NSIP met the UCTD criteria.[12] However, our observations suggest that idiopathic NSIP consisted of two populations with distinct prognoses: patients fulfilling and those not fulfilling the UCTD criteria. Second, the profiles of symptoms and signs associated with CTDs were different. Kinder et al. reported that the most common symptoms and signs were GERD (65%) and arthralgias/joint swelling (64%). However, skin change and Raynaud's phenomenon were most

Table 4 Pathological and radiologic findings of NSIP patients who fulfill the criteria for undifferentiated connective tissue disease compared with those who do not.

Features	UCTD-NSIP[a]	Non-UCTD-NSIP[b]	*P* value
Pathological findings			
Cellular NSIP/Fibrotic NSIP	2/20	3/22	n.s.
Alveolar wall inflammation	2.0 ± 0.4[c]	1.9 ± 0.6	n.s.
Intra-alveolar macrophages	0.8 ± 0.4	0.8 ± 0.8	n.s.
Organizing pneumonia	0.8 ± 0.8	0.6 ± 0.7	n.s.
Germinal center	0.7 ± 1.2	0.3 ± 0.5	n.s.
Fibrosis	2.2 ± 0.6	2.2 ± 0.8	n.s.
Honey combing	0.3 ± 0.8	0.6 ± 0.3	n.s.
Pleural lesion	0.3 ± 0.9	0.3 ± 0.9	n.s.
Radiologic findings			
Ground-glass attenuation, %	95.5	92.0	n.s.
Airspace consolidation, %	68.2	40.0	0.0534
Interlobular septal thickening, %	22.7	40.0	n.s.
Intralobular reticular opacity, %	77.2	72.0	n.s.
Thickening of bronchovascular bundles, %	63.6	36.0	0.0586
Traction bronchiectasis, %	95.5	96.0	n.s.
Honeycombing, %	4.5	12.0	n.s.
Cysts, %	4.5	8.0	n.s.
Lower zone predominance, %	77.3	68.0	n.s.

UCTD, undifferentiated connective tissue disease; n.s., not significant.
[a] NSIP patients who fulfill the criteria for UCTD.
[b] NSIP patients who do not fulfill the criteria for UCTD.
[c] Scores 0-3, Mean ± SD.

frequently present in our patients with UCTD-NSIP (50% and 41%, respectively). The reason for these discrepancies is unknown. The study of Kinder et al. included only 17 patients with idiopathic NSIP, whereas we examined a relatively large number of patients (47 cases). In addition, there was a wide ethnic dissimilarity between the two studies. These differences in study population may be partly related to the discrepancies. To resolve this issue, future studies on a larger series of patients with idiopathic NSIP are necessary.

Because the disease entity of UCTD has not been fully established and there has been no validation of the criteria of Kinder et al., interpretations of our observations and those of Kinder et al. should be made with great caution. Initially, UCTD was defined as systemic autoimmune disorders with signs and symptoms that do not sufficiently fulfill the accepted classification criteria for the defined CTDs.[13] Thus, UCTD was also considered a latent or subclinical phase of the defined CTDs, developing overt CTDs later.[19,20] However, Mosca et al. demonstrated that only a small population of patients with UCTD developed the defined CTDs, in particular, early in their clinical course.[15–17] Thus, they concluded that UCTD is a clinical entity distinct from other defined CTDs. In contrast, other studies reported the relatively high prevalences (35–68%) of progression to

Table 5 Treatment and outcome of NSIP patients who fulfill the criteria for undifferentiated connective tissue disease compared with those who do not.

	UCTD-NSIP[a] (n = 22)	Non-UCTD-NSIP[b] (n = 25)	*P* value
Treatment, n (%)			
Corticosteroids alone	6 (36)	10 (40)	n.s.
Corticosteroids + immunosuppressive agents	9 (41)	10 (40)	n.s.
Cyclosporine	6	7	
Cyclophosphamide	2	1	
Azathioprine	1	2	
Duration of therapy, yr	3.5 (0.6–15.2)[c]	4.0 (1.2–13.6)	n.s.
Death due to respiratory failure, n (%)	1 (5)	8 (32)	0.0170

n.s., not significant.
[a] NSIP patients who fulfill the criteria for UCTD.
[b] NSIP patients who do not fulfill the criteria for UCTD.
[c] Mean ± SD.

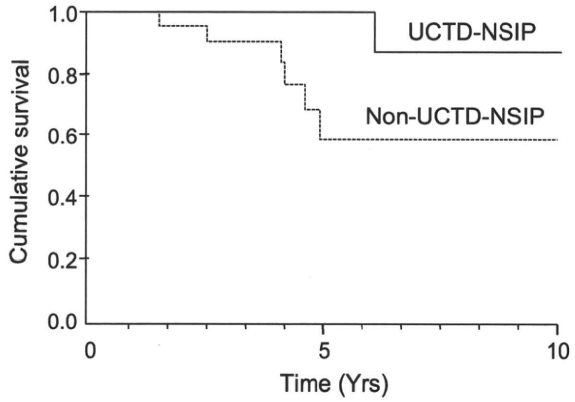

Figure 1 Survival curves of NSIP patients. Patients fulfilling the criteria of undifferentiated connective tissue disease (UCTD-NSIP) have a significantly better survival rate than those who do not fulfill (Non-UCTD-NSIP) (log-rank, $p = 0.0092$).

defined CTDs during the first and second years of follow-up in patients with UCTD.[14,19,21–23] Considering these conditions, Mosca et al. proposed criteria for UCTD as follows: (1) signs and symptoms suggestive of a CTD, but not fulfilling the criteria for any defined CTDs, (2) positive antinuclear antibodies, and (3) a disease duration of at least 3 years.[24] Importantly, the criteria of Kinder et al. used in the present study have several differences, compared with those of Mosca et al. First, the criteria of Kinder et al. does not include disease duration. As a result, patients with the defined CTDs that had not fulfilled the criteria for the defined CTDs at the initial visit, but that met the criteria of Kinder et al. for UCTD, are incorrectly diagnosed as UCTD. In those patients, anti-inflammatory and immunosuppressive treatment for NSIP often masks the later development of the overt defined CTDs. Second, although the criteria of Kinder et al. included sedimentation rate (>two times normal) and C-reactive protein as evidence of systemic inflammation (Table 1), these measurements are highly non-specific. Third, similarly, GERD listed in symptoms associated with CTDs (Table 1), which was the most common symptom in the study of Kinder et al., is not specific for CTDs. Further studies will be needed to fully define UCTD and to develop validated criteria for this disease. At present, it is, at least, true that the criteria of Kinder et al. pick out patients who have symptoms and/or signs that are suggestive of autoimmune disorders. In this context, an alternative implication of our results is that a part, but not all, of patients with idiopathic NSIP had CTD-like features but did not fulfill the criteria for the defined CTDs, and that those patients with CTD-like features showed distinct favorable prognosis. Possibly, idiopathic NSIP with CTD-like features may include true UCTD and early phases of the defined CTDs. Indeed, six (9.7%) of our 62 patents initially diagnosed with idiopathic NSIP progressed to PM/DM during the observation periods, and all the six patients had fulfilled the UCTD criteria but had not met the PM/DM criteria at the first visit.

Besides the lack of validation of the UCTD criteria we used, there are several other limitations to the present study. First, this was a retrospective study, so there were selection and recall biases. Second, although the present study included a relatively large number of patients with idiopathic NSIP, the sample size was still too small to determine the precise prevalence and clinical characteristics of those who meet the UCTD criteria.

In conclusion, we showed that idiopathic NSIP included subjects who fulfilled the UCTD criteria proposed by Kinder et al. Additionally, subjects fulfilling the UCTD criteria had different clinical characteristics with significantly better outcome than those who did not meet the criteria. These data suggest that a part of patients with idiopathic NSIP showed CTD-like features with a distinct prognosis. Future studies will be required to validate our observations.

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Conflict of interest statement

None of the authors has declared any conflict of interest related to this work.

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総　説

薬疹はどうして起こるか
—薬疹発症メカニズムの不思議—

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要　旨

薬疹は，薬剤をハプテンとした抗原認識によって生じるアレルギー反応として単純にとらえられない。特定のVβ鎖を有したT細胞が薬剤に対して反応しやすいこと，また，MHC非拘束性の薬剤によるT細胞反応が存在することなど，ハプテン化抗原認識反応だけでは説明できないからである。そこに混在するのは，Pichlerらの提唱するpharmacological interaction conceptに相当する，必ずしも薬剤感作を必要としない，非特異的なメモリーT細胞活性化反応である。したがって，薬疹の発症は，メモリーT細胞に発現するT細胞受容体と薬剤との親和性に依存し，そのレパートアを規定すると考えられる感染症の経験に左右されると想像される。薬疹がウィルス発疹と酷似するのは，ウィルス抗原によって感作されたメモリーT細胞が，薬剤による非特異的活性化反応を起こしたからかもしれない。

(J Environ Dermatol Cutan Allergol, 4 (2) : 67-75, 2010)

キーワード：薬疹，ウィルス発疹，MHC，拘束性，T細胞受容体

はじめに

「なぜ私はこの病気にかかってしまったのでしょうか。」

よくこのような質問を受ける。帯状疱疹や溶連菌感染症などの病原体による疾病の場合は，比較的答えやすく，患者も理解してくれる。一方，膠原病やアトピー性皮膚炎などの慢性疾患の場合は，説明する医師は苦労を強いられるが，幸運なことに，執拗な追求に難渋することはまれである。さて，なぜ私は薬疹になったのですかと聞かれた時，この質問に対し毅然として答えられる医師はいるのだろうか。

薬疹は原因と結果が明快である。しかし，一見単純に思えるそのプロセスは決して単純ではないことが，近年の研究で明らかとなってきた。薬疹は医原性疾患である。だからこそ臨床家はこの疾患について常に注意をはらわなければならないし，最もその臨床をよく知る皮膚科医は，その機序を明らかにし，臨床に還元する使命があると私は思う。今回は，これまで知られている薬疹の発症メカニズムについて解説しながら，薬疹の発症機序を推理し，今後の展望について述べる。

薬疹の常識

ほとんどの薬疹はT細胞が関与する免疫反応である。患者に限らず，薬疹に関してあまり詳しくないものは皮疹が生じた前日に内服した薬剤を原因薬と信じている場合が多いが，実際には，原因薬でない場合が多い。一般に皮疹の出現には，T細胞が薬剤に対して感作されるに必要な期間，すなわち通常は7日から14日間，平均9日間が必要とされる。実験的な抗原の感作期間は100時間といわれていることから想像しても，7～14日間という期間は妥当な期間と思われる。翻って，発症日から逆算して原因薬剤を類推することも，臨床では多い。ただし，発

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Table 1：Patient profiles (Ref. 2)

Pts	Age/Sex	Type[i]	Duration[ii]	Fever[iii]	Liver enzyme (AST/ALT, IU/l)	Eosinophils (/ml)	CRP (mg/dl)	dLST[iv] (S.I.)
A	21/M	TEN	25	+	53/121	1600	10.9	3.68
B	73/M	SJS	13	+	26/54	100	4	3.55
C	51/M	SJS	14	+	53/52	1000	8.3	10.19
D	24/M	SJS	23	+	26/38	400	0.1	1.2
E	2/M	MPE	8	+	Aug-60	50	0.7	3.43
F	75/M	MPE	Not known	-	Not done	Not done	Not done	Not done
G	34/F	MPE	Not known	-	Not done	Not done	Not done	2.2
H	74/F	MPE	10	-	47/32	500	0.5	1.88
I	51/F	MPE	14	-	32/30	400	0.1	1.82
J	68/M	Fixed	92	-	30/22	700	1.1	Not done

i) Type of clinical manifestation is shown as: TEN, toxic epidermal necrolysis; SJS, Stevens-Johnson syndrome; MPE, maculo-papular eruption and Fixed, fixed drug eruption.
ii) Interval between drug intake and eruption (days) is indicated.
iii) Body temperature > 38 ℃ is indicated as fever +.
iv) Lymphocyte stimulation test using phenobarbital. Stimulation indexes (S.I.) are shown.

症まで極端に短い場合や，薬剤誘発性過敏症候群(drug-induced hypersensitivity syndrome, DIHS)に代表されるように，内服から発症まで数ヵ月を要する場合などの例外がある[1]ことを，認識する必要はある。同一薬剤であっても，臨床型には多様性がある。確かに重症薬疹の原因薬は比較的限られているようであるが，個々の症例をみると，必ずしも薬剤代謝に関する肝や腎の負担の大小や，薬剤そのものの毒性の強弱に薬疹発症の有無は左右されない。すなわち，胃薬やビタミン薬など比較的"体に優しい"薬剤であっても，薬疹の発症に限っていえば，決して起こさないと断言はできない。したがって，原因と結果が明確である場合は，診断が容易であるが，多剤を内服している患者に起こる皮疹について，この薬剤が原因であると断言できるだけの根拠を得ることはむずかしく，診断は困難な場合が多い。

同じ薬剤でなぜ臨床型が異なるのか

同じ薬剤による薬疹でも症状の軽重や臨床像に違いがあるのはなぜだろうか。私たちはさまざまな臨床型の10例のphenobarbitalによる薬疹患者を経験し，その患者の末梢血を用いて，薬剤反応性のT細胞の特徴を検討した[2](Table 1)。Stevens-Johnson症候群（SJS）/中毒性表皮壊死融解症(TEN)患者は4名，maculo-papular eruption(MPE)患者は5名で固定薬疹型の患者が1名であったが，重症例ではCRP値，肝酵素値がより高かった。臨床症状の激しい例の薬剤リンパ球刺激試験は全身症状が乏しいものに比べてその値が有意に高い（Stimulation index, 5.8 ± 3.8 vs 2.3 ± 0.75, $p < 0.05$）ことから，薬剤反応性T細胞の頻度と重症度には相関があると考えられた。

各臨床型において皮疹部組織の浸潤細胞のフェノタイプを調べると，表皮細胞障害の強さに応じて，CD8陽性細胞の浸潤の程度が強い傾向にあった。水疱内容を解析し得た1例のSJS/TENでは，浸潤細胞の多くが活性化抗原であるCD69やCD56を発現するCD8陽性細胞が圧倒的に多かったことも，表皮障害と本細胞との強い関連を示唆させる（Fig. 1)。また，これらの患者の血液からphenobarbital反応性T細胞クローン/ラインを樹立すると，表皮細胞障害が強い症例においてはCD8陽性細胞が多かった。CD8陽性細胞は，perforin, granzyme, granulysinなどの細胞傷害性蛋白，Fas-リガンドおよび可溶性Fasリガンドなどアポトーシス誘導に関する分子を発現/産生し，直接または間接的に細胞傷害性に機能する。臨床的重症度，特に皮膚科にて最も問題とされる表皮の障害の程度は，反応する