

## Subacute and Chronic Prurigo Effectively Treated with Recombinant Interferon- $\gamma$ : Implications for Participation of Th2 Cells in the Pathogenesis of Prurigo

YOSHIKI TOKURA<sup>1</sup>, HIROAKI YAGI<sup>1</sup>, KOTARO HANAOKA<sup>1</sup>, TAISUKE ITO<sup>1</sup>, KYOKO TOWYAMA<sup>1</sup>, YOSHIFUMI SACHI<sup>2</sup>, MASATO TANAKA<sup>3</sup>, TAIKO SAKAMOTO<sup>3</sup>, TOSHIYUKI SEKIZUKA<sup>1</sup>, FUKUMI FURUKAWA<sup>1</sup> and MASAHIRO TAKIGAWA<sup>1</sup>

<sup>1</sup>Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, <sup>2</sup>Section of Dermatology, Hamaoka Municipal Hospital, Hamaoka, and <sup>3</sup>Section of Dermatology, Fujieda Municipal General Hospital, Fujieda, Japan

Subacute and chronic prurigo is notoriously resistant to usual therapies. Four of five patients with a subacute or chronic form of prurigo responded well to daily intravenous injections of recombinant interferon- $\gamma$  (rIFN- $\gamma$ ) (0.25–2  $\times$  10<sup>6</sup> Japan Reference Unit (JRU; 1 JRU roughly corresponds to 4 NIH units) daily, for 10–14 days). In one patient examined, the dermal portion of lesional skin before the treatment contained considerable amounts of mRNA for interleukin (IL)-4, IL-5, and IL-10, indicative of infiltration of Th2 cells. Furthermore, the administration of rIFN- $\gamma$  selectively down-regulated mRNA for Th2 cytokines, IL-4 and IL-10 in peripheral blood mononuclear cells. These findings suggest that Th2 cells play a pathogenetic role in these types of prurigo and that rIFN- $\gamma$  exerts its efficacy by inhibiting Th2 cells. Our pilot study suggests that the systemic administration of rIFN- $\gamma$  is a therapeutic alternative for the treatment of recalcitrant prurigo. **Key words:** cytokine; therapy; T-lymphocytes.

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Y. Tokura, Department of Dermatology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431–31, Japan.

According to the tentative classification proposed by European dermatologists, prurigos are divided into acute, subacute and chronic forms (1–3). In acute prurigo, the stings of insects are thought to cause the primary lesions. Subacute prurigo tends to affect middle-aged women, and its lesions are symmetrically distributed (1, 3). The primary lesion preceding excoriation has been proved in patients with subacute prurigo (4). The etiology of this type of prurigo is variable (2, 5). Chronic prurigo is a poorly-defined entity, which occurs in adults with a peak incidence between the ages of 40 and 60 (3). The eruption consists of small, irritable papules whose course may be continuous for months or years (3). Chronic prurigo is occasionally associated with malignant diseases such as Hodgkin's disease and polycythemia (3). Subacute and chronic forms of prurigo have been treated with various modalities, including oral antihistamines, psychopharmaceuticals, tranquilizers, topical corticosteroids and emollients (1). However, the disease is notoriously resistant to these therapies.

On the basis of distinct patterns of cytokine secretion, T-helper cell populations are categorized into the Th1 cell subset secreting interleukin (IL)-2 and interferon- $\gamma$  (IFN- $\gamma$ ), and the Th2 cell subset producing IL-4, IL-5, and IL-10 (6–8). These two types of T-cells and their cytokines counter-regulate the biological behavior of each other (6). A preliminary study

showed that the lesional dermis of subacute prurigo expressed mRNA for IL-4, IL-5, and IL-10, suggesting that Th2 cells are involved in the development of prurigo. Therefore, we decided to treat patients with subacute or chronic prurigo with the systemic administration of recombinant IFN- $\gamma$  (rIFN- $\gamma$ ). In this article, we report that 4 of 5 patients with prurigo, who had responded poorly to the usual therapeutic regimens, were effectively treated with rIFN- $\gamma$ .

### MATERIALS AND METHODS

#### Patients

Five patients (aged 42–70 years; 3 subacute and 2 chronic forms), with symptoms for 4 months to 9 years, are reported. The diagnoses of subacute and chronic forms were made based on the Burton's description (3). Briefly, papulonodules are distributed symmetrically on the extremities and trunk in the subacute form, and the chronic form consists of relatively small papules. Prurigo nodularis was not included in this study. The patients had earlier been treated with antihistamines and topical corticosteroids. Two cases had also received psoralen and ultraviolet A (PUVA) therapy. Skin lesions in the 5 patients had been recalcitrant to or merely partially improved by former therapies. All patients except one subacute case showed blood eosinophilia (>500/cmm) and 2 subacute cases exhibited high levels of IgE (1,430 and 2,350 IU/ml; normal, <400 IU/ml). None of the cases had a history of atopic disease or a positive RAST reaction to *Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*. None of the patients had internal malignancies. Biopsy specimens of skin lesions in all cases showed a perivascular infiltrate of lymphoid cells in the upper dermis, with mild spongiform epidermis. Eosinophils were occasionally intermingled with lymphoid cells in cases 1 to 3.

#### Evaluation of therapeutic effects of rIFN- $\gamma$

Treatments and all examinations in this study were performed after informed consent had been obtained. The patients had not received any systemic corticosteroids or psoralen and ultraviolet A (PUVA) therapy for at least 2 months before the study. Oral antihistamines and topical corticosteroids, if administered, were continued without changing their regimens. rIFN- $\gamma$  (Biogamma, Maruho Co., Osaka, Japan, and Suntory Ltd, Osaka, Japan; specific activity, 5  $\times$  10<sup>6</sup> Japan Reference Unit (JRU)/mg of protein; 1 JRU roughly corresponds to 4 NIH units) was given intravenously (i.v.). The rIFN- $\gamma$  dose (x10<sup>6</sup> JRU daily) was 0.25–2 for the first 7–19 days and 0.5–2 twice weekly for 2 months in cases 1 and 2. Cases 3 and 4 were treated daily for 10 days (total dose, 17–20) and case 5 for 7 days (total dose, 7). Pruritus and skin eruption were graded as 3, 2, 1, and 0. For pruritus, the gradation represented severe (3), moderate (2), mild (1), and none (0). Skin eruption was classified into erythematous/highly raised (3), erythematous/moderately raised (2), erythematous/flattened (1), and pigmentation/depigmentation alone (0). The total clinical severity score (0–6) was defined as the sum of the individual scores of these two parameters. The clinical efficacy of rIFN- $\gamma$  administration was evaluated by comparing the severity of skin lesions before and 7 to

14 days after administration of rIFN- $\gamma$ . Peripheral leukocyte counts with differential percentages and IgE levels were examined periodically.

*Analysis of cytokine mRNA expression by peripheral blood mononuclear cells (PBMC) and lesional skin*

Total RNA was prepared from PBMC and the dermal portion of the skin lesion, as described previously (9, 10). Reverse transcription (RT) to cDNA and polymerase chain reaction (PCR) amplification were performed with the Takara RNA PCR kit (GeneAmp RNA PCR Kit; Takara Biomedicals, Osaka, Japan) according to the manufacturer's directions, using specific primers for IL-2, IL-4, IL-5, IL-10, IFN- $\gamma$  and  $\beta$ -actin, as described previously (9, 10). After PCR products were separated by electrophoresis on 2% agarose, the gel was stained with 1  $\mu$ g/ml ethidium bromide, and the amplified DNA bands were visualized in an ultraviolet transilluminator. The presence of cytokine-specific mRNA transcripts was confirmed by visual inspection as the presence of bands having the predicted number of base pairs, with the use of a DNA molecular weight marker VI (Boehringer Mannheim GmbH, Mannheim, Germany) electrophoresed in parallel.

## RESULTS

### *Therapeutic effects of rIFN- $\gamma$*

The administration of rIFN- $\gamma$  has been approved by the Ministry of Health and Welfare in Japan for the treatment of patients with cutaneous T-cell lymphoma, which is performed usually with  $2 \times 10^6$  JRU daily for several weeks. When administered i.v., rIFN- $\gamma$  causes flue-like symptoms such as headache, fever and myalgia (11). Therefore, diclofenac sodium (25 mg) was occasionally given 1 h before the injection to minimize side effects. The tolerated dose was determined by gradual daily elevation of the dose in cases 1, 4, and 5. Fig. 1 illustrates changes in the total severity score. Cases 1 to 4 responded well to rIFN- $\gamma$ , as the severity score was reduced from 6 or 5 of pretreated values to 3 within 7 days and further declined to 1 or 2 by 14 days after rIFN- $\gamma$  administration, whereas case 5 (chronic) was not changed after a 7-day administration.

Since the discontinuation of rIFN- $\gamma$  increased the severity score, the daily administration was followed by twice weekly usage in cases 1 and 2, which resulted in the complete absence of active lesions in case 1 (severity score, 0) and persistent but mild lesions in case 2 (severity score, 3). After the cessation of rIFN- $\gamma$  administration, eruptions were well-controlled with

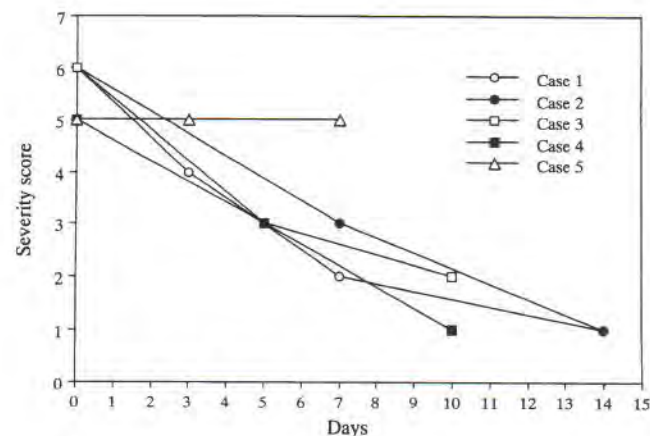


Fig. 1. Changes in the total severity score before and after rIFN- $\gamma$  administration in 5 patients.

an oral antihistamine and topical corticosteroids in case 3, and marked exacerbation of eruptions was seen and the severity score of the eruption returned to the pre-treated level in case 4. In these 2 patients, weekly injections of rIFN- $\gamma$  for maintenance were not performed. Liver and renal functions, and the blood electrolyte levels had been normal in all patients during the treatment and 6 months' follow-up.

### *Changes in eosinophil number and IgE level*

After a 7- to 14-day administration of rIFN- $\gamma$ , the number of blood eosinophils was decreased by  $\sim 60\%$  in 2 (cases 2 and 3) of 4 cases that had shown eosinophilia before treatment. In cases 1 and 5, the eosinophil count was not substantially changed. In case 1, however, a long-term follow-up revealed that circulating eosinophils were reduced in number following discontinuation of rIFN- $\gamma$  administration, as described below. In case 4 without eosinophilia, the number of eosinophils was slightly increased after rIFN- $\gamma$  injections.

Case 1 was followed up for long periods both before and after rIFN- $\gamma$  treatment in regard to the number of eosinophils and level of IgE (Fig. 2). These two parameters had increased gradually during 10 months until rIFN- $\gamma$  was given. Eosinophils fluctuated in number during daily injections of rIFN- $\gamma$ , with relatively stable levels of IgE. After the regimen was changed to twice weekly injection, the level of IgE was elevated transiently and decreased thereafter. Following complete disappearance of active prurigo lesions, both eosinophil counts and IgE levels were gradually normalized.

### *Cytokine mRNA expression by dermal infiltrating cells and circulating lymphocytes in case 1*

Fig. 3 shows cytokine mRNA expression in a biopsy specimen of lesional skin in case 1. The dermal portion of the lesional skin contained detectable amounts of mRNA for IL-2, IL-4, IL-5, IL-10 and IFN- $\gamma$ . Since dermal samples from normal control subjects express mRNA for IL-2 and IFN- $\gamma$  but not for IL-4 or IL-5 (10), the expression of mRNA in the lesional skin skewed to a Th2 cytokine profile.

On analysis of cytokine mRNA expression by freshly isolated PBMC from case 1, mRNA for IL-2, IL-4, IL-10, but not IL-5, was detected by RT-PCR before the treatment. When the patient had been treated with daily i.v. injection of rIFN- $\gamma$  for 5 consecutive days, the PCR products for IL-4 and

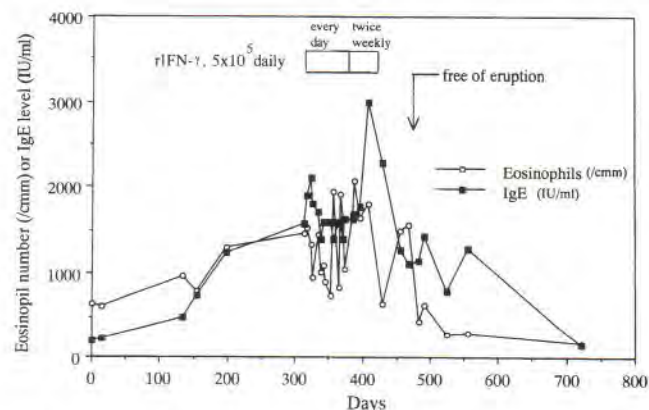


Fig. 2. Changes in the number of eosinophils and level of IgE in case 1.

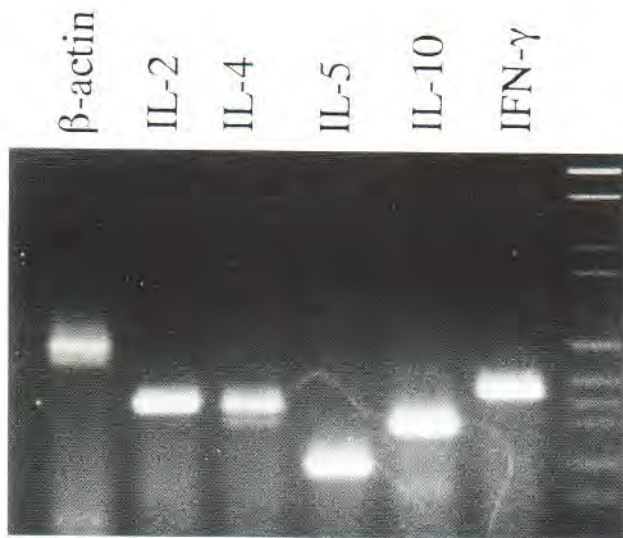


Fig. 3. Cytokine mRNA expression by dermal infiltrating cells in case 1. RNA extracted from lesional dermis was analyzed. The sizes of predicted base pairs were 661 for  $\beta$ -actin, 458 for IL-2, 456 for IL-4, 291 for IL-5, 396 for IL-10 and 501 for IFN- $\gamma$ .

IL-10 were markedly and moderately decreased, respectively, whereas the message for IL-2 was increased by the treatment (Fig. 4). Thus, rIFN- $\gamma$  down-regulated mRNA for Th2 cytokines and simultaneously up-regulated mRNA for IL-2.

#### DISCUSSION

In this study, 4 of 5 patients with subacute and chronic forms of prurigo responded well to daily administration of rIFN- $\gamma$ . Since these patients were recalcitrant to oral antihistamines, topical corticosteroids and PUVA therapy, it was considered that rIFN- $\gamma$  was beneficial for the treatment of subacute and chronic prurigos. It was especially intriguing that the successive twice weekly administration of rIFN- $\gamma$  resulted in disappearance of active lesions in case 1. However, since this is an

uncontrolled pilot study, we cannot exclude the possibility that a substantial placebo effect on pruritus reduced scratching, resulting in improvement of skin lesions.

In contrast to undetectable levels of mRNA expression for IL-4 or IL-5 in dermis from normal control subjects (10), the lesional dermis of case 1 contained considerable amounts of PCR products for these cytokines, suggesting that the eruption was due to a Th2-type reaction. Furthermore, a selective effect of rIFN- $\gamma$  on the cytokine transcription was found in PBMC of case 1: injections of rIFN- $\gamma$  down-regulated mRNA for Th2 cytokines, IL-4 and IL-10, and simultaneously up-regulated mRNA for IL-2. These findings suggest that Th2 cells participate in the pathogenesis of these types of prurigo and that rIFN- $\gamma$  exerts its therapeutic efficacy by inhibiting Th2-mediated functions. Although it has been reported that serum IgE levels are not changed by rIFN- $\gamma$  administration (12), case 1 suggests that a decrease in serum IgE may occur when patients are followed up for a longer period, even after the cessation of the administration.

Prurigo is a widely used dermatologic term without an internationally satisfactory definition, which causes breakdown in communication, especially between North American and non-American dermatologists (1). The terminological confusion may be caused by lack of agreement as to whether the pruritic papule or nodule is a primary lesion or a secondary lesion resulting from excoriation. Presumably because of its confused definition, the mechanisms of prurigo have been poorly investigated. Our study implies that some subacute and chronic forms of prurigo are at least partly immunologically mediated by Th2 cells. In this context, it is interesting that some patients with subacute prurigo have an atopic background (5) and that pruriginous lesions were historically described in association with atopic dermatitis, such as Besnier's and Hebra's prurigos (3). Given a Th2 nature of the immunologic state in atopic dermatitis (13), our study raises the possibility that subacute and chronic prurigos share immunologic conditions with atopic dermatitis even in patients lacking atopic histories. It has been reported that in patients with prurigo nodularis, which some authors believe to be an extreme case of prurigo chronica (1), early-onset prurigo is closely associated with an atopic nature (14). This appears to be in accordance with the finding that the onset ages of cases 1 and 2, showing a good therapeutic response to rIFN- $\gamma$ , are 46 and 33 years and that eruptions first appeared at 66 years in case 5, with a poor response. In fact, rIFN- $\gamma$  has also been tried for the treatment of atopic dermatitis (12). However, according to our preliminary study using the same protocol of rIFN- $\gamma$  administration, the therapeutic efficacy of rIFN- $\gamma$  is better in prurigo than atopic dermatitis.

The administration of rIFN- $\gamma$  is expected to improve disorders in which Th2 cells are pathogenic by counteracting Th2 cells and activating Th1 cells. These disorders include cutaneous T-cell lymphoma, whose malignant cells are of a Th2 nature (10), eosinophilic pustular folliculitis (11), Wells' syndrome (Yagi et al., manuscript submitted for publication), and atopic dermatitis (12). In addition, rIFN- $\gamma$  may be a therapeutic choice for the treatment of certain types of recalcitrant prurigo.

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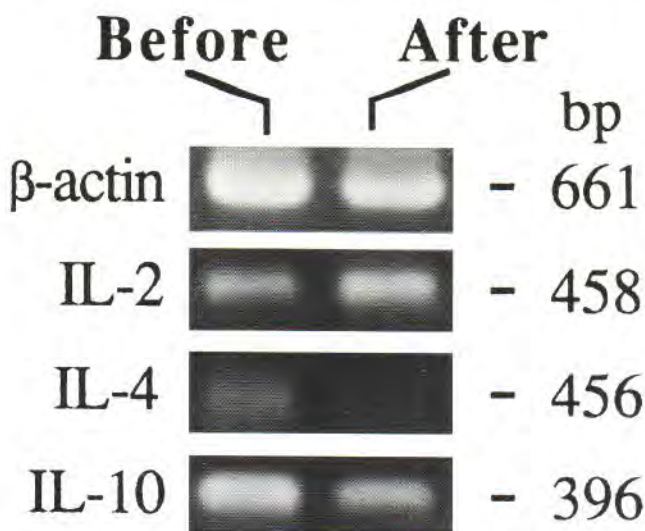


Fig. 4. Changes in cytokine mRNA expression of freshly isolated PBMC from case 1. RNAs extracted from PBMC taken from the patient before and after 5 days of rIFN- $\gamma$  administration were analyzed.

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