DNA vaccination against macrophage migration inhibitory factor improves atopic dermatitis in murine models

Asuka Hamasaka, MD,[a] Riichiro Abe, MD, PhD,[a] Yoshikazu Koyama, PhD,[b] Naoya Yoshioka, MS,[a] Yasuyuki Fujita, MD,[a] Daichi Hoshina, MD,[a] Mikako Sasaki, MS,[a] Tsutomu Hirasawa, PhD,[c] Shin Onodera, MD, PhD,[d] Shigeki Ohshima, MD, PhD,[e] Lin Leng, PhD,[f] Richard Bucala, MD, PhD,[f] Jun Nishihira, MD, PhD,[b] Tadamichi Shimizu, MD, PhD,[g] and Hiroshi Shimizu, MD, PhD[a] *Sapporo, Ebetsu, Osaka, and Toyama, Japan, and New Haven, Conn*

Background: Atopic dermatitis (AD) is a common chronic inflammatory skin disease. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that has been implicated in the pathogenesis of AD. Recently, we developed a novel DNA vaccine that generates neutralizing endogenous anti-MIF antibodies.

Objective: This study explores the preventive and therapeutic effects of this MIF-DNA vaccine in mouse models of AD.

Methods: Two different AD model mice (DS-Nh and NC/Nga) received MIF-DNA vaccination to analyze preventive and therapeutic effects, as assessed by clinical skin scores, histologic findings, and serum IgE levels.

Results: In murine models of AD, MIF-DNA vaccination prevented the occurrence of the AD skin phenotype. Furthermore, administration of MIF-DNA vaccine to mice that had already developed AD produced a rapid improvement in AD skin manifestation. There were reduced histologic signs of inflammation and lower serum IgE levels in treated mice compared with those seen in control animals. Finally, passive transfer of IgG from MIF-DNA vaccinated mice to AD mice also produced a significant therapeutic effect. These results demonstrate that MIF-DNA vaccination not only prevents the development of AD but also improves the symptoms of pre-existing AD.

Conclusion: Taken together, the induction of an anti-MIF autoantibody response using MIF-DNA vaccination appears to be a useful approach in the treatment of AD. (J Allergy Clin Immunol 2009;124:90-9.)

Key words: *Atopic dermatitis, macrophage migration inhibitory factor, DNA vaccination*

From the Departments of [a]Dermatology, [d]Sports Medicine and Joint Reconstruction Surgery, and [e]Orthopedics, Hokkaido University Graduate School of Medicine, Sapporo; [b]the Department of Medical Information, Hokkaido Information University, Ebetsu; [c]Discovery Research Laboratories, Shionogi and Co Ltd, Osaka; [f]the Department of Medicine, and Pathology, Yale University School of Medicine, New Haven; and [g]the Department of Dermatology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama.

Disclosure of potential conflict of interest: R. Bucala receives grant support from the National Institutes of Health, the Alliance for Lupus Research, and Promedior. The rest of the authors have declared that they have no conflict of interest.

Received for publication November 5, 2008; revised April 13, 2009; accepted for publication April 14, 2009.

Available online June 1, 2009.

Reprint requests: Riichiro Abe, MD, PhD, or Hiroshi Shimizu, MD, PhD, Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan. E-mail: aberi@med.hokudai.ac.jp and shimizu@med.hokudai.ac.jp.

0091-6749/$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.04.025

Abbreviations used

- AD: Atopic dermatitis
- MIF: Macrophage migration inhibitory factor
- TTX: Tetanus toxin P30 T_H epitope

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease with significant morbidity and an adverse effect on patient well-being.[1] The prevalence of AD has increased 2- to 3-fold during the past 3 decades in industrialized countries, and it presently occurs in 10% to 20% of children and 1% to 3% of adults.[2] AD is thought to result from a dysregulation in the normal interaction between the environment, genes, defects in skin barrier function, and systemic and local immunologic responses.[3] The contribution of the immune response to the pathogenesis of AD has been largely attributed to abnormalities in the adaptive immune system, with key roles played by T_H1/T_H2 cell dysregulation, IgE production, dendritic cell signaling, and mast cell hyperactivity, leading to the pruritic inflammatory dermatosis that characterizes AD.[3]

Macrophage migration inhibitory factor (MIF) is an upstream regulator of the inflammatory response, and it is upregulated in various inflammatory disorders, including AD.[4] We previously reported that serum MIF levels in patients with AD were significantly increased compared with those seen in healthy control subjects and patients without AD.[5] In addition, circulating MIF levels in patients with AD decrease as the clinical features of the disease improve, suggesting that MIF might play a pivotal role in the inflammatory response in these patients.[5,6] Moreover, MIF promotes IL-2 and IL-2 receptor expression and memory T-cell development, and it might influence T_H1/T_H2 cell differentiation responses.[6,7] Based on these observations suggesting that MIF might be a therapeutic target in AD, we hypothesized that inhibition of MIF with neutralizing antibodies might induce beneficial therapeutic effects in patients with AD.

Monoclonal antibodies directed against proinflammatory cytokines, such as TNF-α, have been used for the treatment of rheumatoid arthritis, Crohn disease, and psoriasis,[8-10] and there have been a few reports describing the use of anti–TNF-α mAbs for the treatment of AD.[7,8] The application of mAbs to AD nevertheless might be difficult because of the requirement for frequent injections, the large quantities of immunoglobulin protein required, and the associated costs of production. Moreover, even fully humanized antibodies are potentially immunogenic and might elicit antibody responses, thereby limiting their long-term therapeutic efficacy. These limitations have led to the development of alternative neutralization strategies, including methods that aim to elicit autoantibodies against target proteins,

such as cytokines or pathogens, by administering them in a naked or partially modified form as therapeutic vaccines.

We recently developed an MIF-DNA vaccine that breaks immunologic tolerance by introducing oligonucleotides encoding a foreign T_H cell epitope into the murine MIF cDNA sequence.[11,12] We demonstrated that this MIF-DNA vaccination elicits production of endogenous anti-MIF antibodies and showed a significant amelioration of symptoms in murine models of rheumatoid arthritis[9] and sepsis.[10]

The present study describes for the first time the preventive and therapeutic effects of this MIF-DNA vaccine in 2 different mouse models of AD.

METHODS

Animals

Six-week-old female BALB/c mice were purchased from Japan Clea (Shizuoka, Japan). Male DS-Nh mice were provided by Aburabi Laboratories, Shionogi and Co, Ltd (Shiga, Japan), and male NC/Nga mice were purchased from SLC (Hamamatsu, Japan). All mice were bred and housed under conventional conditions, and procedures were conducted according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee under an approved protocol.

Production of DNA vaccine

We previously reported the design of the MIF/tetanus toxin P30 T_H epitope (TTX) DNA expression plasmid and our analysis of the *in vitro* expression of MIF/TTX by using this plasmid.[11] For the generation of immunologically active MIF antigen, an MIF construct harboring a T_H epitope at its second loop region was designed. For that purpose, the coding region for the second loop of the mouse MIF, amino acids 32 to 37 (GKPAQY), was deleted from the MIF cDNA and substituted with an *Eco*RI site. A complementary DNA coding for the TTX (FNNFTVSFWLRVPKVSASHL) with *Eco*RI sites at both termini was obtained by means of hybridization of partially overlapping oligo DNAs (sense, ggaattcaacaacttcaccgtgagcttctggctgcgcgtgcccaa; antisense, ggaattccaggtggctggcgctcaccttgggcacgcgcagccaga) after polymerization with the Klenow fragment of DNA polymerase. After digestion with *Eco*RI, the cDNA coding for the P30 T_H epitope was inserted into the *Eco*RI site of the MIF expression plasmid lacking the second loop, and a clone with the insert of correct orientation was selected. For vaccination, the plasmid DNA was purified by using standard methods with alkaline lysis followed by 2 rounds of CsCl density gradient ultracentrifugation.

Vaccination protocols

Gene transfer into muscle by means of electroporation was performed as described previously.[11] Briefly, mice were anesthetized with ether and shaved near their hind legs. A pair of electrode needles (5-mm gap and 0.5-mm diameter; NEPA GENE, Chiba, Japan) was then inserted into an anterior tibial muscle, and DNA vaccine (25 μg/25 μL of 0.9% saline) was injected into the portion between the needles. Electrical pulses (50 V, 50 ms, 3 times) were applied (T820 and Optimizer 500; BTX, San Diego, Calif) and followed by another 3 pulses with inverted polarity. The same injection and electroporation was applied to the other tibial muscle. Thus 50 μg of the naked plasmid was injected per mouse into the tibias. A similar vaccination was repeated 3 weeks later.

Evaluation of anti-MIF antibody titer in sera of DNA-vaccinated mice

Anti-MIF titers in plasma were determined by means of direct ELISA. Briefly, individual plasma from vaccinated mice were collected from the tail vein and diluted with 0.1% BSA/PBS/0.05% Tween 20. Small aliquots of diluted plasma (1:200) were added into 96-well flat-bottom plates precoated with recombinant MIF. Anti-MIF antibodies that reacted with the precoated recombinant MIF were detected with goat anti-mouse antibody conjugated with horseradish peroxidase, followed by color development with substrate reagent (Techne, Minneapolis, Minn).

Evaluation of clinical skin severity score

Mice were macroscopically observed and scored by 2 persons blind to the treatment protocol. Before skin conditions were scored, scratching behavior was observed for 2 minutes. A total clinical severity for AD-like lesions was defined as the sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of 5 signs and symptoms (itch, erythema, edema, excoriation/erosion, and scaling/dryness).[13]

Measurement of IgE and TNF-α levels in sera

Serum total IgE levels were measured by using a sandwich ELISA kit (Yamasa Shouyu, Chiba, Japan). Serum MIF levels were assayed with ELISA kits for Genetic Lab (Sapporo, Japan). The ELISA procedures were conducted according to the manufacturer's instructions. The concentration of TNF-α was determined by using the BD Cytometric Bead Array (BD PharMingen, San Jose, Calif). Flow cytometric analysis was carried out with a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, Calif).

Real-time PCR analysis

Total RNA was extracted from dorsal skin to quantify cytokine mRNA expression levels in dermatitis lesions. RNA samples were analyzed with the ABI prism 7000 sequence detection system (Applied Biosystems, Foster City, Calif). Primers and probes specific for IL-1β, IL-4, IL-6, and IFN-γ were obtained from the TaqMan gene expression assay (Applied Biosystems). Differences between the mean cycle threshold (CT) values of cytokines and those of β-actin (Applied Biosystems) were calculated as

$$\Delta CT_{sample} = CT_{cytokine} - CT_{\beta-actin},$$

and those of ΔCT for the normal adult skin were calculated as

$$\Delta CT_{calibrator} = CT_{cytokine} - CT_{\beta-actin}.$$

Final results for fetal skin sample/adult skin (as percentages) were determined as $2-(\Delta CT_{sample} - \Delta CT_{calibrator})$.

Histologic analysis

Six-micrometer-thick sections of dorsal skin were stained with hematoxylin and eosin, acidic toluidine blue (pH 4.0) for mast cells, and direct fast scarlet for eosinophils. Cells between the epithelium and panniculus carnosus were counted at a magnification of ×400 and were expressed as the total number of cells in 5 fields.

Treatment of neutralizing MIF mAbs

Neutralizing anti-MIF mAb (NIH-III.D9) was previously described.[14] Neutralizing MIF mAbs (50 μg) or control IgG (50 μg) were injected intravenously into 15-week-old NC/Nga mice with dermatitis twice a week for 3 weeks.

Adoptive transfer of autoantibodies elicited by DNA vaccines

IgG was purified from the sera of control pCAGGS plasmid– or MIF/TTX-vaccinated DS mice at 6 weeks after the vaccination by using the protein A Antibody Purification Kit (Amersham Biosciences, Piscataway, NJ). The purified IgG was tested for its ability to suppress ongoing dermatitis in an adoptive transfer experiment. DS-Nh mice with developing dermatitis were separated at 15 weeks of age into 3 equally sick groups of 3 mice each. Every 3 days, these animals were administered 50 μg per mouse of purified IgG from control pCAGGS plasmid–vaccinated DS-Nh mice, purified IgG from MIF/TTX-vaccinated DS-Nh mice, or an equal volume of PBS.

RESULTS

MIF/TTX vaccination prevents the onset of AD in DS-Nh mice

DS-Nh mice housed under conventional conditions but not in a specific pathogen free environment spontaneously exhibit AD-like skin symptoms, including erythema, edema, excoriation, erosion, dry skin, and desquamation.[15-17] Early skin symptoms appear around 9 weeks of age and continue to worsen until age 25 weeks. An increase in total serum IgE levels is detected at approximately 17 weeks of age and after the development of skin lesions.[15-17]

We first examined the potential protective effect of the MIF/TTX vaccine on dermatitis development by treating 9-week-old DS-Nh mice before the development of skin eruptions. The clinical features of the control pCAGGS plasmid–vaccinated mice were similar to those of untreated mice. At 18 weeks of age, or 9 weeks after the vaccination, both groups of mice showed severe erythema, erosions, and dry skin (Fig 1, *A*). By contrast, the MIF/TTX-vaccinated mice exhibited almost no eruptions (Fig 1, *B*). The clinical skin score of MIF/TTX-vaccinated mice was low until 21 weeks of age (Fig 1, *C*), which is a time at which the MIF/TTX-vaccinated mice showed high serum levels of anti-MIF antibodies (Fig 1, *D*). Furthermore, in the MIF/TTX-vaccinated mice the serum level of IgE was significantly decreased and the serum MIF level was only slightly decreased when compared with those seen in the control vaccinated mice (Fig 1, *E* and *F*). In addition, cytokine expression in affected skin lesions was analyzed by using real-time PCR. The T_H2 cytokine IL-4 was very slightly downregulated, and the T_H1 cytokine IFN-γ was slightly upregulated. Of note, the expression of the proinflammatory cytokines IL-1β and IL-6 was significantly suppressed in MIF-vaccinated mice compared with that seen in control mice (Fig 1, *G*). Therefore the inhibition of MIF in the atopic model mice appears to result primarily in the suppression of inflammation rather than affecting the T_H1/T_H2 cytokine balance.

Improvement of clinical skin condition with MIF/TTX vaccine also was confirmed by the observation that the lesions of mice vaccinated with MIF/TTX vaccine showed amelioration in hyperkeratosis, acanthosis, dermal edema, and infiltration of the inflammatory cells at 21 weeks when compared with the condition of mice vaccinated with control pCAGGS plasmid (Fig 2, *A*). At the affected skin sites, the numbers of eosinophils and mast cells decreased significantly in the MIF/TTX-vaccinated mice at 21 weeks when compared with those seen in the control vaccinated mice (Fig 2, *B-D*).

These data clearly show that MIF/TTX vaccination can prevent the onset of AD-like dermatitis in DS-Nh mice.

MIF/TTX vaccination improves pre-existing AD

To determine whether the MIF/TTX vaccine has any therapeutic effect in AD, we next vaccinated 15-week-old DS-Nh mice with pre-existing AD and evaluated the progression of skin changes. Mice treated with the control plasmid continued to exhibit severe dermatitis 6 weeks after vaccination (Fig 3, *A*). By contrast, the MIF/TTX vaccination significantly improved dermatitis symptoms (Fig 3, *B*). The clinical skin scores of control-vaccinated mice increased after the vaccination, whereas that of MIF/TTX-vaccinated mice began to decrease at 21 weeks of age (Fig 3, *C*). At this time, the MIF/TTX-vaccinated mice showed high levels of anti-MIF antibodies (Fig 3, *D*). Furthermore, the serum IgE and MIF levels of MIF/TTX-vaccinated mice also were lower than those of control mice at 21 weeks of age (Fig 3, *E* and *F*). In addition, serum TNF-α levels were significantly lower in the MIF/TTX-vaccinated mice when compared with those seen in the control plasmid–vaccinated mice (Fig 3, *G*).

By means of histologic analysis, the lesions of mice vaccinated with MIF/TTX vaccine showed improvement of hyperkeratosis, acanthosis, dermal edema, and infiltration of inflammatory cells at 21 weeks when compared with the control plasmid–vaccinated mice (Fig 4, *A*). In addition, the numbers of eosinophils and mast cells decreased significantly in the MIF/TTX-vaccinated mice at 21 weeks when compared with those seen in the control mice (Fig 4, *B-D*). The serum IgE level of MIF/TTX-vaccinated mice decreased at 21 weeks of age compared with that of control-vaccinated mice (Fig 4, *E*).

These data indicate that MIF/TTX vaccination leads to an improvement in already established dermatitis in the DS-Nh mice.

We further observed that MIF-DNA vaccine improved the manifestation of pre-existing AD in a second model of AD, which develops in the NC/Nga strain.[11,13] We vaccinated 15-week-old NC/Nga mice with dermatitis, and although the control pCAGGS plasmid–vaccinated mice still had severe dermatitis 6 weeks after the vaccination treatment (Fig 5, *A*), the MIF/TTX-vaccinated mice showed significant improvement (Fig 5, *B*). The clinical skin score of control-vaccinated mice increased after the vaccination, whereas that of MIF/TTX-vaccinated mice began to decrease at 21 weeks of age (Fig 5, *C*). At this time, the MIF/TTX-vaccinated mice showed high levels of anti-MIF antibodies (Fig 5, *D*). The serum IgE and MIF levels of MIF/TTX-vaccinated mice decreased at 21 weeks of age (Fig 5, *E* and *F*). These data show that MIF-DNA vaccination improves dermatitis not only in the DS-Nh strain but also in the NC/Nga mouse strains.

To confirm that anti-MIF antibodies suppress AD, we performed an additional therapeutic experiment by using a neutralizing anti-MIF mAb. Anti-MIF mAb (50 μg) or an isotypic control IgG (50 μg) were injected intravenously into 15-week-old NC/Nga mice with dermatitis twice a week for 3 weeks. Anti-MIF mAbs, as well MIF vaccination, significantly improved AD skin manifestations when compared with conditions seen in control IgG–treated mice (Fig 5, *G* and *H*).

Adoptive transfer of autoantibodies elicited by MIF/TTX-DNA vaccine suppressed AD

To better substantiate that the therapeutic action of MIF/TTX-DNA vaccination could be attributed to anti-MIF autoantibodies, we performed adoptive transfer of purified IgG from vaccinated DS-Nh mice into naive DS-Nh mice. The purified IgG was adoptively transferred into the 15-week-old DS-Nh mice that had already demonstrated skin eruptions. As shown in Fig 6, this IgG was effective in ameliorating AD, indicating that the therapeutic effect of MIF/TTX vaccination could be adoptively transferred by immune serum IgG.

DISCUSSION

In the present study we have shown that active vaccination against MIF is a novel preventive and therapeutic approach in 2 murine models of AD. We showed that mice administered a MIF/TTX-DNA vaccine did not experience the cutaneous manifestations of AD. The MIF/TTX-DNA vaccine also improved the symptoms

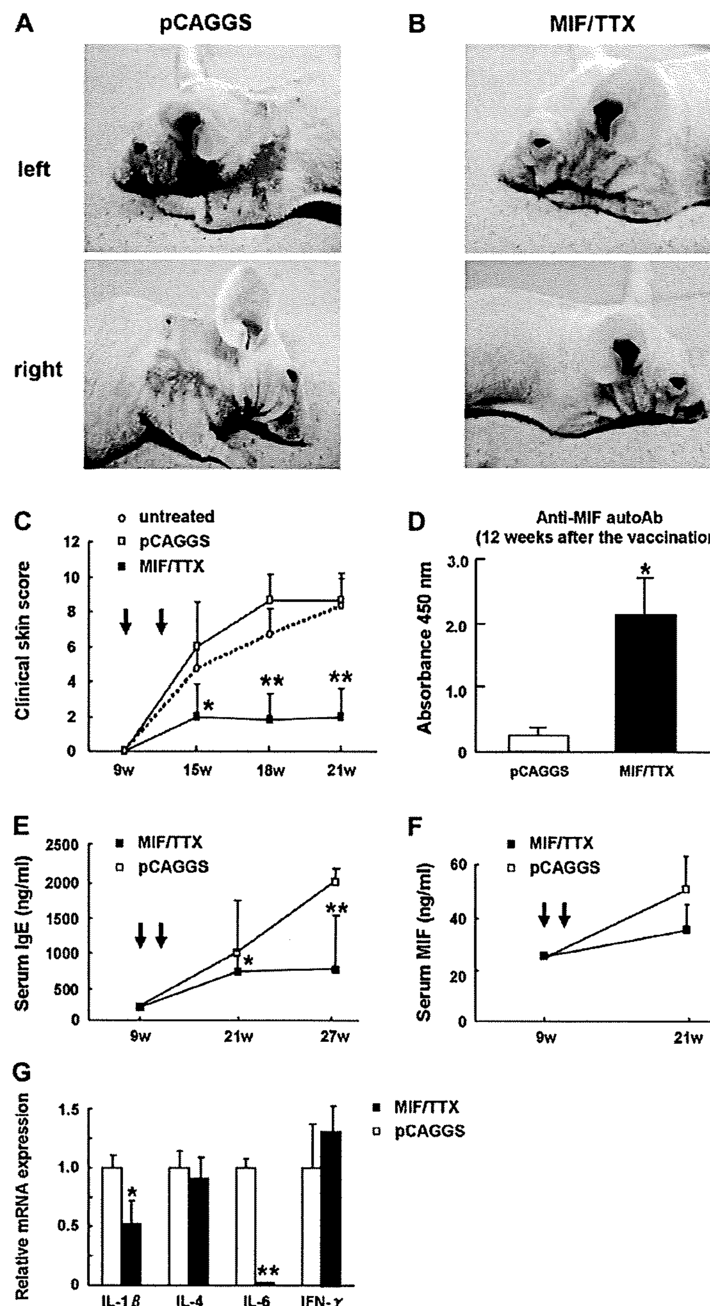


FIG 1. Prevention of the onset of AD by MIF/TTX-DNA vaccine. Nine-week-old DS-Nh mice without skin eruptions were subjected to administration of MIF/TTX or a control plasmid (pCAGGS). Clinical features of 21-week-old DS-Nh mice vaccinated with endotoxin-free pCAGGS **(A)** and MIF/TTX (**B**; 12 weeks after the vaccination) are shown. **C,** The clinical skin score of mice immunized with the MIF/TTX-DNA vaccine *(solid squares)*, immunized with pCAGGS plasmid *(open squares)*, or left untreated *(open circles)*. Results are given as means ± SEs of 5 mice in each group. $*P < .01$ and $**P < .005$ versus pCAGGS at the same time point. **D,** Serum level of anti-MIF autoantibodies *(autoAb)* at 12 weeks after vaccination. Means ± SEs are shown (n = 5). $*P < .01$. **E,** Serum IgE levels of the mice vaccinated with MIF/TTX *(solid squares)* and pCAGGS *(open squares)*. $*P < .01$ and $**P < .005$ for MIF/TTX versus pCAGGS at the same time point. Means ± SEs of 5 mice in each group are shown. **F,** Serum MIF levels of the mice vaccinated with MIF/TTX *(solid squares)* and pCAGGS *(open squares)*. **G,** Cytokine expression (IL-1β, IL-4, IL-6, and IFN-γ) in affected skin lesions was analyzed by using real-time PCR. $*P < .05$ and $**P < .001$.

of pre-existing AD in 2 different strains of AD-prone mice, the DS-Nh and NC/Nga strains. Finally, we demonstrated that the therapeutic effect of MIF/TTX vaccination could be adoptively transferred by serum IgG that contained MIF autoantibodies.

Proinflammatory cytokines are believed to be important contributors to the pathogenesis of skin inflammation in patients with AD, which might depend on the duration of the skin lesion. Patients with acute AD typically have a systemic T_H2 response

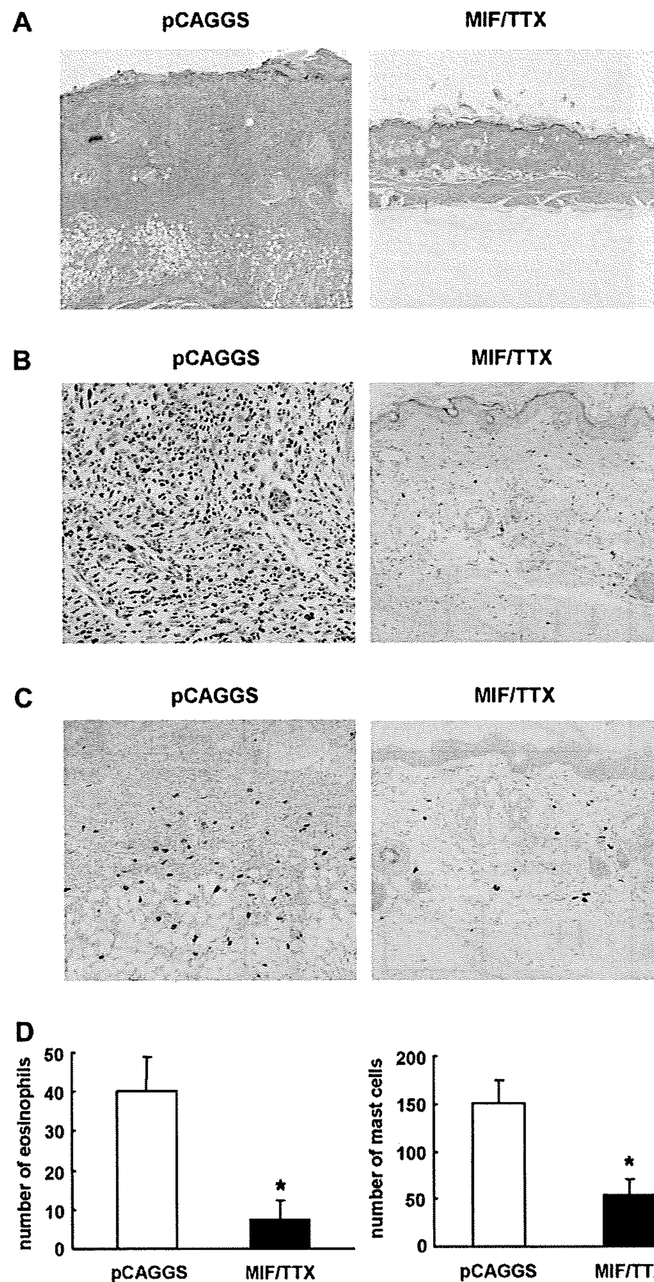


FIG 2. Histologic analysis of AD in DS-Nh mice vaccinated with MIF/TTX or pCAGGS plasmid before disease onset. Nine-week-old DS-Nh mice without eruptions were administered MIF/TTX or control plasmid. Specimens were collected from the dorsal skin 6 weeks after the first vaccination and stained with hematoxylin and eosin (**A**, original magnification ×40), direct fast scarlet for eosinophils (**B**, original magnification ×200), or toluidine blue for mast cells (**C**, original magnification ×200). **D**, The number of eosinophils and mast cells in 5 high-power fields from 4 individual skin specimens were enumerated by means of microscopy. Means ± SEs of 4 mice are shown. $*P < .001$ for MIF/TTX versus pCAGGS.

with increased serum IgE levels, eosinophilia, and a marked infiltration of T_H2 cells into acute skin lesions. The infiltrating T cells show a predominance of IL-4, IL-5, IL-10, and IL-13 expression.[12,18] In patients with chronic AD, however, there is infiltration of eosinophils and macrophages, and the disease becomes associated with an increase in the expression of IL-12, with a switch to T_H1 cellular responses.[12,18] Chronic AD skin lesions in adults with a prolonged duration of disease have been shown to manifest an increase in the expression of IL-1, IL-5, IL-12, IFN-γ, TNF-α, GM-CSF, and MIF.[12,18] This biphasic T_H1/T_H2 switch in immune response is characterized pathologically by lichenification, epidermal hyperplasia, and dermal fibrosis. MIF regulates the production of various proinflammatory cytokines, including TNF-α, and the inflammatory cytokines in response to stimulation by LPS are known to be suppressed in MIF-deficient mice. We previously reported that MIF-deficient mice have an impaired contact hypersensitivity (CH) response and that immunoneutralization of MIF effectively suppresses CH response[19]; these observations led us to speculate that MIF would be a therapeutic target for AD.

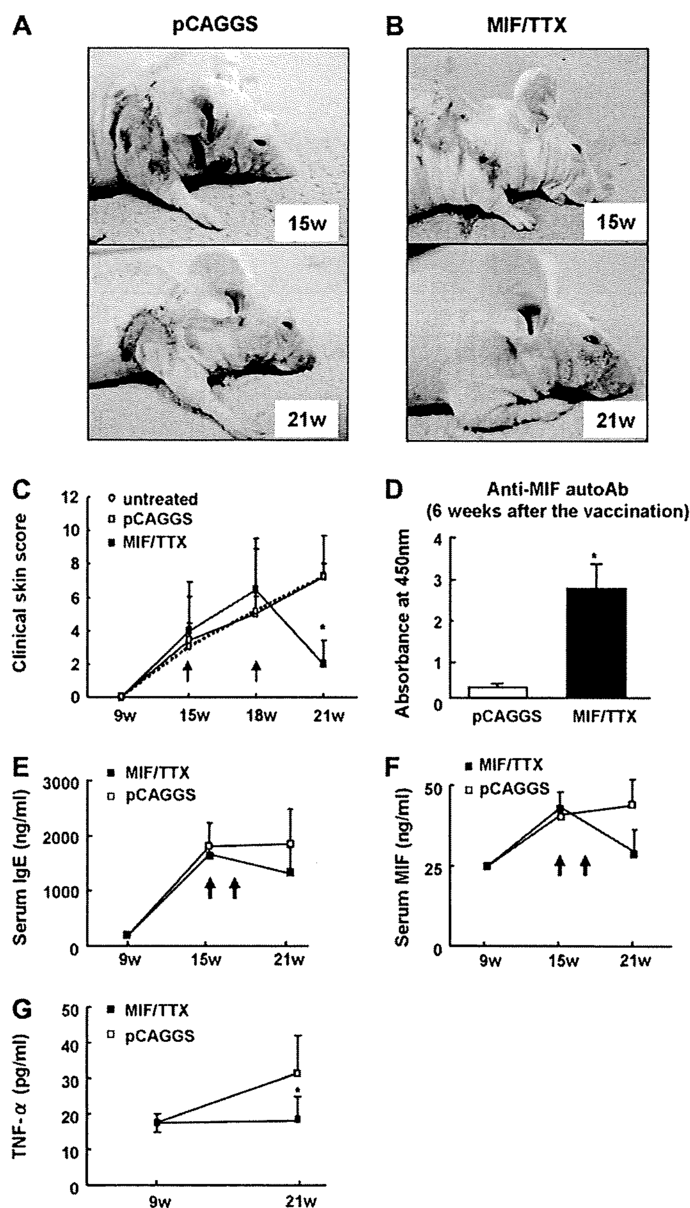


FIG 3. Therapeutic effect of MIF-DNA vaccination in DS-Nh mice with pre-existing AD. Fifteen-week-old DS-Nh mice with ongoing dermatitis were administered MIF/TTX or control plasmid (pCAGGS) or left untreated. **A,** Clinical features of DS-Nh mice vaccinated with control plasmid. **B,** Clinical features of DS-Nh mice vaccinated with MIF/TTX. **C,** Clinical skin scores of mice immunized with MIF/TTX-DNA vaccine *(solid squares)*, control plasmid *(open squares)*, or left untreated *(open circles)*. Means ± SEs of 10 mice per group are shown. $*P < .005$ for MIF/TTX versus pCAGGS. **D,** Serum level of anti-MIF autoantibodies *(autoAb)* at 6 weeks after the vaccination. Means ± SEs are shown (n = 10). $*P < .001$. Serum IgE **(E)** and MIF **(F)** levels of the mice vaccinated with MIF/TTX vaccine *(solid squares)* and control pCAGGS plasmid *(open squares)* are shown. The data shown are for 10 mice per group. **G,** The serum levels of TNF-α were decreased in MIF/TTX-vaccinated mice *(solid squares)* compared with those seen in the control (pCAGGS) plasmid–vaccinated mice *(open squares)*. $*P < .01$ for MIF/TTX versus pCAGGS.

The therapeutic aim of cytokine vaccine therapy is to induce high titers of circulating polyclonal autoantibodies to neutralize the pathologic levels of a particular cytokine. The advantages of this therapy include the potential to maintain high antibody titers, long-term efficacy, and low cost. Monoclonal antibodies directed against TNF-α have been used for the treatment of psoriasis.[10] Jacobi et al[7] recently reported a clinical trial of infliximab monotherapy for 9 patients with moderate or severe AD who showed significant improvement in all clinical parameters; however, this improvement was not sustained by maintenance of the therapy. The authors considered that the development of antichimeric antibodies could explain the lack of a durable response to infliximab maintenance therapy. A cytokine vaccine results in the production of native antibodies, and it might overcome this limitation in anticytokine antibody therapy.

It is unknown whether long-term inhibition of MIF activity might be safe in human subjects. A major limitation of an active immunization approach is the inability to control the outcome. However, it should be noted that serum MIF levels in the MIF-DNA–vaccinated mice were maintained at a baseline level

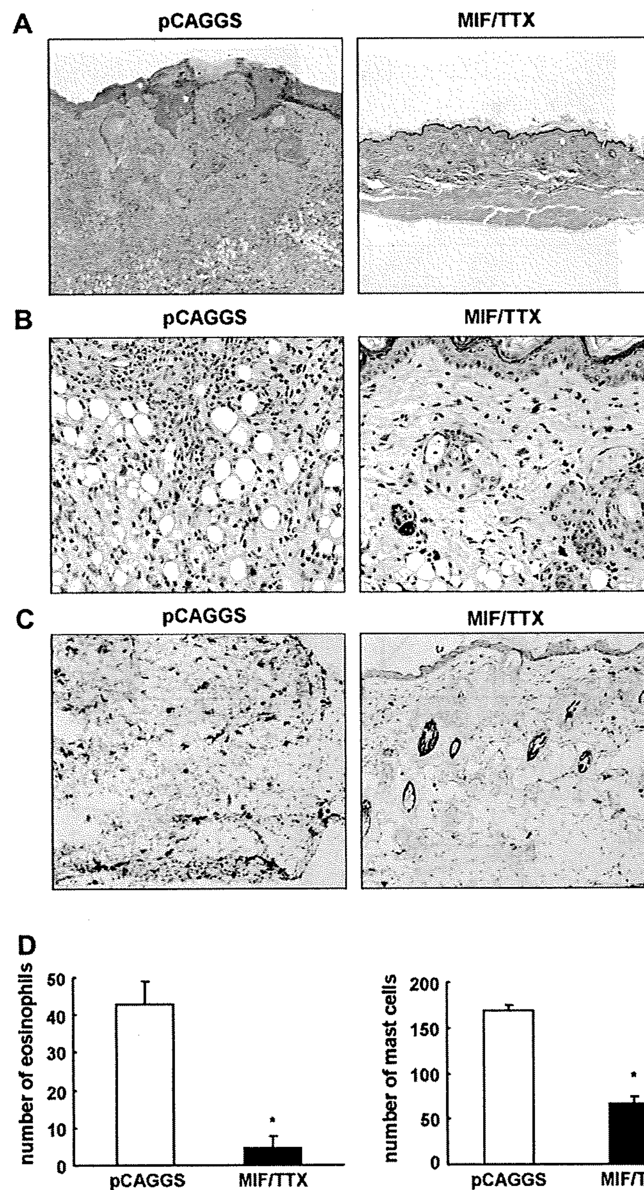


FIG 4. Histologic analysis of AD in DS-Nh mice vaccinated with MIF/TTX or control plasmid after disease onset. Fifteen-week-old DS-Nh mice with ongoing dermatitis were administered MIF/TTX or control (pCAGGS) plasmid. Specimens were collected at 6 weeks after the first vaccination and stained with hematoxylin and eosin (**A**, original magnification ×40), direct fast scarlet for eosinophils (**B**, original magnification ×200), or toluidine blue for mast cells (**C**, original magnification ×200). **D**, The number of eosinophils and mast cells in 5 high-power fields from 4 individual skin specimens were enumerated by means of microscopy. Data represent means ± SEs of 4 mice. *$P < .001$ for MIF/TTX versus pCAGGS.

(Figs 2, *F*, and 4, *F*), despite an anti-MIF antibody level that remained high for 6 weeks of vaccine administration. It is possible that the present protocol of vaccination dose not induce a high enough level of anti-MIF antibody to inhibit serum MIF protein completely. There are reports that autoantibody production induced by vaccine-encoded antigens regress to baseline levels shortly after remission in acute experimental autoimmune encephalomyelitis,[15] whereas in adjuvant-induced arthritis[16] autoantibodies continue to be produced at high titer. It has been considered that targeted DNA vaccines amplify a pre-existing anti–self-regulatory response that by itself is capable of limiting, although not preventing, the emerging autoimmune condition.[15-17,20-22] In addition, we observed that MIF-DNA–vaccinated mice did not show serious side effects, such as evident infections.

Serum IgE levels were significantly decreased when MIF vaccination was used as a preventive agent, whereas the levels were not significantly decreased when it was used as a therapeutic agent. It is known, however, that IgE levels do not parallel the clinical severity of AD in human patients.[19]

The cardinal principles in the treatment of AD are to reduce symptoms, prevent exacerbations, and minimize medication side effects. This approach incorporates the use of emollients, topical corticosteroids, topical calcineurin inhibitors, antihistamines, stress management, and avoidance of allergens or

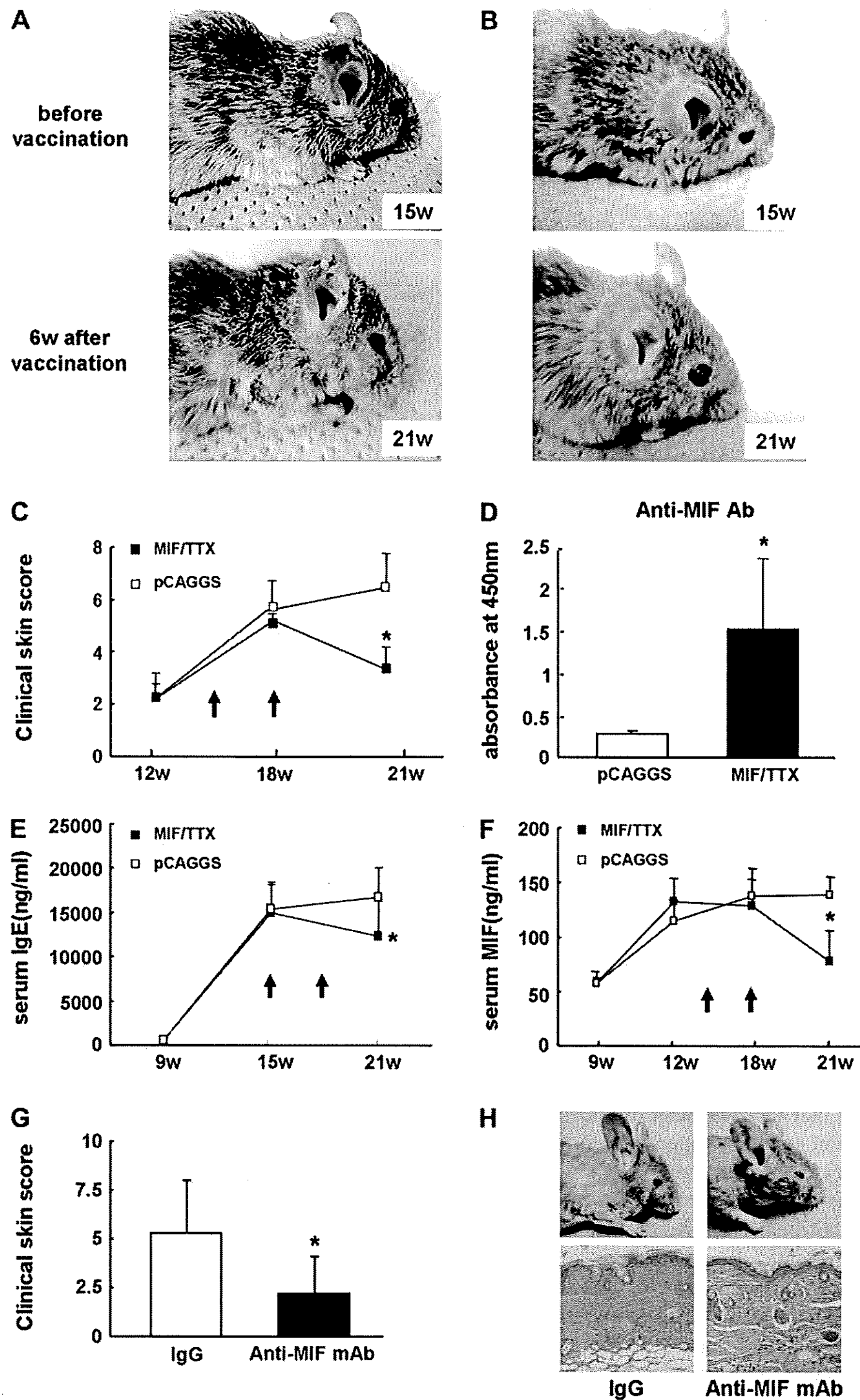


FIG 5. Therapeutic effect of MIF/TTX-DNA in vaccine for pre-existing AD on NC/Nga mice. Fifteen-week-old NC/Nga mice with dermatitis were vaccinated with control pCAGGS plasmid or MIF/TTX. **A,** Clinical features of NC/Nga mice vaccinated with control pCAGGS plasmid. **B,** Clinical features of DS-Nh mice vaccinated with MIF/TTX (*top*, vaccination). **C,** Clinical skin scores of mice vaccinated with MIF/TTX *(solid squares)* and control pCAGGS plasmid *(open squares)*. $*P < .05$ for MIF/TTX versus pCAGGS at the same time point. Each point represents means ± SEs of 10 mice in each group. **D,** Serum level of anti-MIF autoantibodies *(Ab)* at 6 weeks after the vaccination. Means ± SEs are shown (n = 10). $*P < .005$. Serum IgE **(E)** and MIF **(F)** levels of mice vaccinated with MIF/TTX vaccine *(solid squares)* and control pCAGGS plasmid *(open squares)* are shown. Means ± SEs of 10 mice in each group are shown. $*P < .0005$. **G** and **H,** Neutralizing anti-MIF mAbs (50 μg) or control IgG (50 μg) were injected intravenously into 15-week-old NC/Nga mice with dermatitis twice a week for 3 weeks. Fig 5, *G*, shows clinical skin scores ($*P < .05$). In Fig 5, *H*, the *upper panels* show clinical features, and the *lower panels* show histologic images.

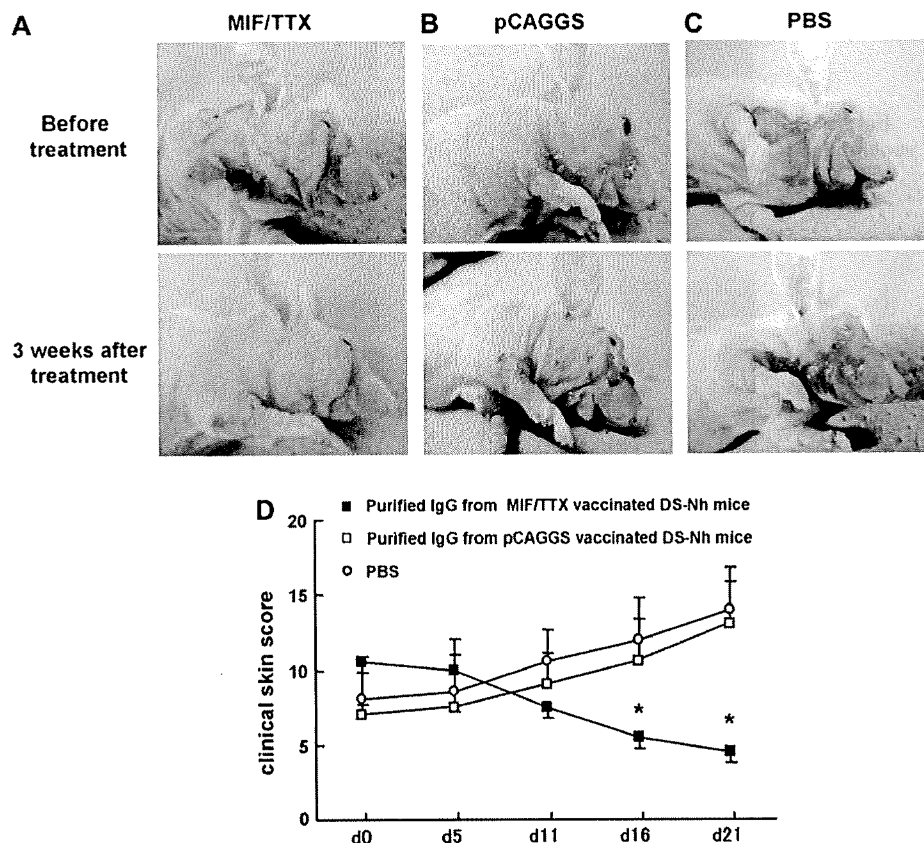


FIG 6. Adoptive transfer of autoantibodies elicited by DNA vaccines. IgG were purified from the sera of control (pCAGGS) plasmid– or MIF/TTX-vaccinated DS mice and adoptively transferred. DS-Nh mice were administered 50 μg per mouse of purified IgG from control plasmid–vaccinated DS-Nh mice, purified IgG from MIF/TTX-vaccinated DS-Nh mice, or PBS. **A-C,** Clinical features at 3 weeks after administration of purified IgG from MIF/TTX-vaccinated mice (Fig 6, *A*), purified IgG from control pCAGGS plasmid–vaccinated mice (Fig 6, *B*), and clinical features after administration of PBS (Fig 6, *C*). **D,** Clinical skin scores of DS-Nh mice administered IgG from MIF/TTX-vaccinated DS-Nh mice *(solid squares)*, IgG from control pCAGGS plasmid–vaccinated DS-Nh mice *(open squares)*, or PBS *(open circles)*. Results are shown as means ± SEs of 3 mice in each group. $*P < .05$ versus pCAGGS at the same time point.

disease triggers.[3] The fact that the treatment of AD mainly depends on the self-application of topical agents often hinders the effective and long-term treatment of the disease. Notwithstanding these conventional treatments, if a patient with severe and refractory AD requires additional therapy, cyclosporine A has been used despite systemic side effects, such as renal toxicity.[23,24] The relapsing and remitting course of AD also places a psychologic, social, and financial burden on patients and their families. New treatment options are needed to prevent the progression of AD to more severe forms of disease and to halt the so-called atopic march toward asthma. An MIF-DNA vaccine approach offers the additional advantage of requiring only a periodic booster injection, and it might allow for the potential resolution of immunopathology in those with chronic refractory disease.

We thank Professor James R. McMillan for his manuscript proofreading and Ms Yuika Osaki for her excellent technical assistance.

Clinical implications: MIF-DNA vaccination might be a useful preventive and therapeutic approach for AD.

REFERENCES

1. Jung T, Stingl G. Atopic dermatitis: therapeutic concepts evolving from new pathophysiologic insights. J Allergy Clin Immunol 2008;122:1074-81.
2. Morar N, Willis-Owen SAG, Moffatt MF, Cookson WOCM. The genetics of atopic dermatitis. J Allergy Clin Immunol 2006;118:24-34.
3. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. J Clin Invest 2004;113:651-7.
4. Shimizu T. Role of macrophage migration inhibitory factor (MIF) in the skin. J Dermatol Sci 2005;37:65-73.
5. Shimizu T, Abe R, Ohkawara A, Mizue Y, Nishihira J. Macrophage migration inhibitory factor is an essential immunoregulatory cytokine in atopic dermatitis. Biochem Biophys Res Commun 1997;240:173-8.
6. Shimizu T, Abe R, Ohkawara A, Nishihira J. Increased production of macrophage migration inhibitory factor by PBMCs of atopic dermatitis. J Allergy Clin Immunol 1999;104:659-64.
7. Jacobi A, Antoni C, Manger B, Schuler G, Hertl M. Infliximab in the treatment of moderate to severe atopic dermatitis. J Am Acad Dermatol 2005;52:522-6.
8. Cassano N, Loconsole F, Coviello C, Vena GA. Infliximab in recalcitrant severe atopic eczema associated with contact allergy. Int J Immunopathol Pharmacol 2006;19:237-40.
9. Onodera S, Ohshima S, Tohyama H, Yasuda K, Nishihira J, Iwakura Y, et al. A novel DNA vaccine targeting macrophage migration inhibitory factor protects joints from inflammation and destruction in murine models of arthritis. Arthritis Rheum 2007;56:521-30.
10. Tohyama S, Onodera S, Tohyama H, Yasuda K, Nishihira J, Mizue Y, et al. A novel DNA vaccine-targeting macrophage migration inhibitory factor improves the survival of mice with sepsis. Gene Ther 2008;15:1513-22.

11. Matsumoto M, Ra C, Kawamoto K, Sato H, Itakura A, Sawada J, et al. IgE Hyperproduction through enhanced tyrosine phosphorylation of Janus kinase 3 in NC/Nga Mice, a model for human atopic dermatitis. J Immunol 1999;162:1056-63.
12. Leung DYM, Bieber T. Atopic dermatitis. Lancet 2003;361:151-60.
13. Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol 1997;9:461-6.
14. Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med 2000;6:164-70.
15. Wildbaum G, Netzer N, Karin N. Plasmid DNA encoding IFN-gamma-inducible protein 10 redirects antigen-specific T cell polarization and suppresses experimental autoimmune encephalomyelitis. J Immunol 2002;168:5885-92.
16. Salomon I, Netzer N, Wildbaum G, Schif-Zuck S, Maor G, Karin N. Targeting the function of IFN-γ-inducible protein 10 suppresses ongoing adjuvant arthritis. J Immunol 2002;169:2685-93.
17. Youssef S, Maor G, Wildbaum G, Grabie N, Gour-Lavie A, Karin N. C-C chemokine-encoding DNA vaccines enhance breakdown of tolerance to their gene products and treat ongoing adjuvant arthritis. J Clin Invest 2000;106:361-71.
18. Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 2000;105:860-76.
19. Abramovits W. Atopic dermatitis. J Am Acad Dermatol 2005;53(suppl):S86-93.
20. Wildbaum G, Youssef S, Karin N. A targeted DNA vaccine augments the natural immune response to self TNF-alpha and suppresses ongoing adjuvant arthritis. J Immunol 2000;165:5860-6.
21. Wildbaum G, Karin N. Augmentation of natural immunity to a pro-inflammatory cytokine (TNF-alpha) by targeted DNA vaccine confers long-lasting resistance to experimental autoimmune encephalomyelitis. Gene Ther 1999;6:1128-38.
22. Youssef S, Wildbaum G, Maor G, Lanir N, Gour-Lavie A, Grabie N, et al. Long-lasting protective immunity to experimental autoimmune encephalomyelitis following vaccination with naked DNA encoding C-C chemokines. J Immunol 1998;161:3870-9.
23. Berth-Jones J, Finlay AY, Zaki I, Tan B, Goodyear H, Lewis-Jones S, et al. Cyclosporine in severe childhood atopic dermatitis: a multicenter study. J Am Acad Dermatol 1996;34:1016-21.
24. Berth-Jones J, Graham-Brown RA, Marks R, Camp RD, English JS, Freeman K, et al. Long-term efficacy and safety of cyclosporin in severe adult atopic dermatitis. Br J Dermatol 1997;136:76-81.

Did you know?

You can now personalize the *JACI* Web site to meet your individual needs. Enjoy these new benefits and more:

- Search across 400 top medical and health sciences journals online, including MEDLINE.
- Greater cross-referencing results from your online searches.

Visit www.jacionline.org today to see what else is new online!

Case Report

Dermatology

Dermatology 2009;218:265–271
DOI: 10.1159/000182268

Received: March 5, 2008
Accepted: August 8, 2008
Published online: December 5, 2008

A Severe and Refractory Case of Anti-p200 Pemphigoid Resulting in Multiple Skin Ulcers and Scar Formation

Y. Goto-Ohguchi[a] W. Nishie[a] M. Akiyama[a] Y. Tateishi[a] S. Aoyagi[a] Y. Tsuji-Abe[a] D. Sawamura[a] N. Ishii[b] T. Hashimoto[b] H. Shimizu[a]

[a]Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, and
[b]Department of Dermatology, Kurume University School of Medicine, Fukuoka, Japan

Key Words
Autoimmune blistering skin disease · Methylprednisolone pulse therapy · Bullous pemphigoid · Type XVII collagen

Abstract
Anti-p200 pemphigoid is a recently described autoimmune blistering skin disease that is characterized by the presence of autoantibodies against an unidentified 200-kDa dermal autoantigen. Most of the previous cases have been successfully treated using mild-to-moderate immunosuppressive therapies, which resulted in a good prognosis. We report here a severe and refractory case of anti-p200 pemphigoid that developed in a 53-year-old woman, in which blisters led to multiple skin ulcers, followed by severe scar formation. In the present case, methylprednisolone pulse therapy was effective enough to reduce the disease activity.
Copyright © 2008 S. Karger AG, Basel

Introduction

Anti-p200 pemphigoid is a recently described autoimmune bullous disease that is characterized by the presence of autoantibodies against an, as yet, unidentified 200-kDa dermal antigen in the lower lamina lucida [1–4]. Most of the patients showed clinical features resembling bullous pemphigoid [1, 2, 5–7], dermatitis herpetiformis [8, 9] or linear immunoglobulin (Ig) A bullous dermatosis [10–12]. In addition, in some patients anti-p200 pemphigoid has been associated with psoriasis [1, 9, 13]. Anti-p200 pemphigoid patients usually show a good response to treatments such as oral corticosteroids [8], minocycline [14], dapsone [6, 10, 15, 16] and cyclosporine [1, 14]. In some cases, even corticosteroid ointment therapy has been used to control the disease [5, 17]. Here, we report a severe and refractory case of anti-p200 pemphigoid, in which the majority of blisters resulted in skin ulcers that spread extensively and scar formation. Interestingly, this patient also had IgG autoantibodies directed against the 180-kDa bullous pemphigoid autoantigen [BPAG2, BP180 or type XVII collagen (Col17)], which might be related to the severity of this case.

Case Report

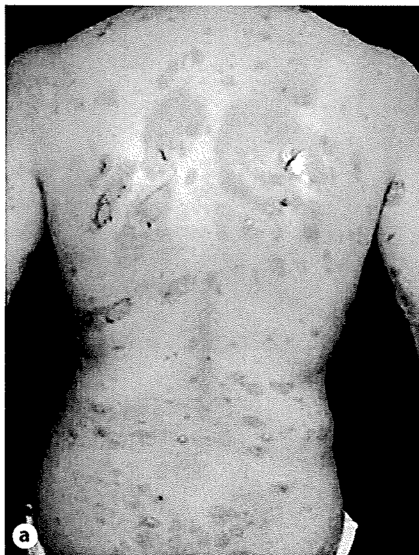
A 53-year-old Japanese female was referred to our outpatient clinic with blisters and erythema on her trunk and extremities, as well as oral and genital mucous membrane involvement. On physical examination, small, tense blisters with pruritic erythema were found over her entire body. The blisters on areas of erythema tended to form in an annular arrangement (fig. 1a–c). The oral and genital mucosa also showed blisters (fig. 1d, e). Histological examination of a skin biopsy specimen taken from the patient's back showed a subepidermal blister with extensive neutrophil and eosinophil infiltration (fig. 2a). Direct immunofluorescence studies revealed linear in vivo deposition of IgG and C3 along the epidermal basement membrane (fig. 2b). Neither IgM nor IgA deposition was detected (not shown). Indirect immunofluorescence studies using as a substrate normal human skin obtained from a healthy volunteer showed a high titer of circulating IgG autoantibodies against the dermal-epidermal junction (over 640-fold dilution). These deposits mapped mostly to the dermal side, and there was also some partial or weak staining to the epidermal side when 1 M NaCl-split human skin was used as a substrate (fig. 2b). Immunoblotting studies revealed that the majority of the circulating IgG autoantibodies were directed against a 200-kDa dermal protein (fig. 2c). In addition, IgG autoantibodies to the noncollagenous 16th-A (NC16A) domain of Col17 were present, demonstrated by immunoblotting using recombinant NC16A domain proteins as a substrate

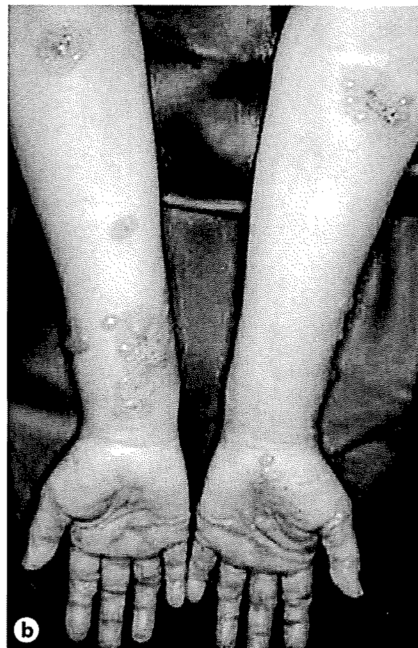
KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

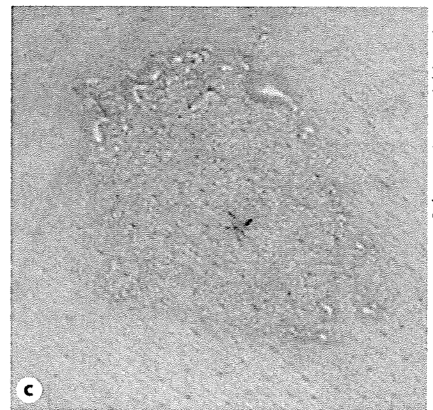
© 2008 S. Karger AG, Basel
1018–8665/09/2183–0265$26.00/0

Accessible online at:
www.karger.com/drm

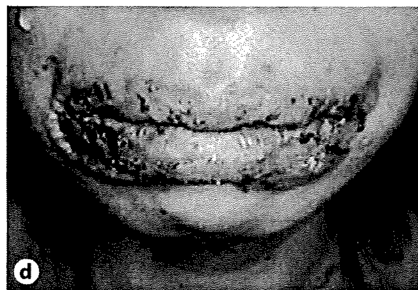
Dr. Wataru Nishie
Department of Dermatology
Hokkaido University Graduate School of Medicine, N15 W7
Sapporo 060-8638 (Japan)
Tel. +81 11 716 1161, Fax +81 11 706 7820, E-Mail nishie@med.hokudai.ac.jp







Color version available online



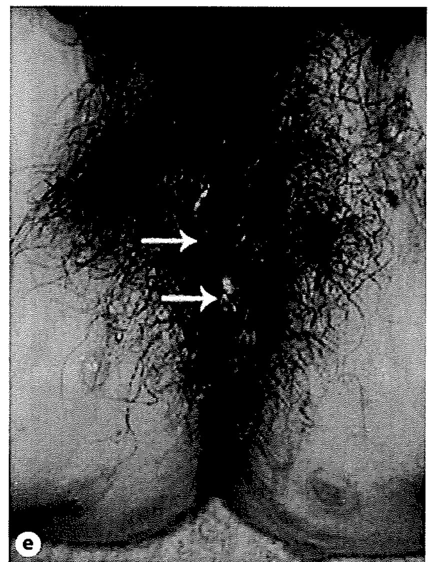


Fig. 1. Clinical features. Small and tense blisters with pruritic erythema were found on the trunk (**a**) and extremities (**b**). Note the blisters on erythema tended to form in an annular arrangement (**c**). The oral (**d**) and genital mucosa (arrows; **e**) were also involved.

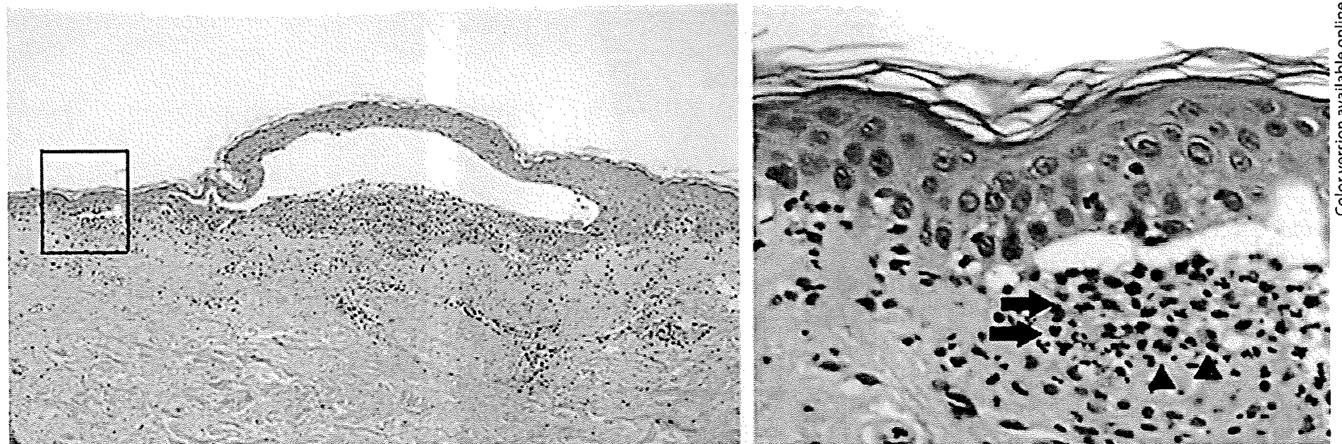
(data not shown), as well as a BP180 ELISA study with an index value of 33.6 (BP180 ELISA kit; MBL, Nagoya, Japan).

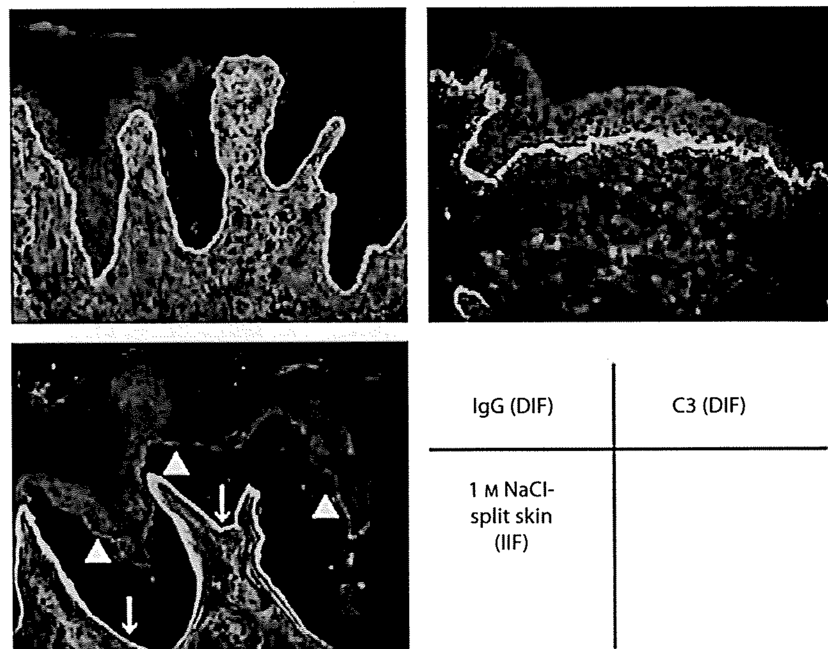
Initially, the patient was treated with 50 mg (1.0 mg/kg) oral prednisolone, 200 mg minocycline and 1,500 mg nicotinamide daily for 14 days. However, new blisters continued to develop and most of these lesions resulted in deep ulcers (fig. 3a). She was then treated with methylprednisolone pulse therapy, 1,000 mg daily for 3 days, which succeeded in reducing the disease activity. The dose of oral prednisolone was then gradually reduced. However, severe scar formation remained on both hands and feet (fig. 3b). Two years after initial treatment, the patient's condition is now controlled with 1 mg oral prednisolone taken daily, and there has been no recurrence of blister formation.

Discussion

Here, we report a severe case of anti-p200 pemphigoid successfully treated with systemic corticosteroids and methylprednisolone pulse therapy. So far, 32 cases of anti-p200 pemphigoid have been reported in the English-language literature (table 1). In previously reported anti-p200 pemphigoid cases, the titers of IgG autoantibodies directed against the dermal-epidermal junction were relatively low (<400-fold dilution) by indirect immunofluorescence [5, 6, 17, 18]. In our current case, the indirect immunofluorescence titer was remarkably high (>640-fold dilution). Although it is still unclear whether the autoantibody titer can be correlated with disease severity in anti-p200 pemphigoid, the antibody titer has been reported to be related to disease activity in other antibody-mediated autoimmune blistering skin diseases [19–21]. Therefore, we propose that the severe clinical manifestations, including wide-spread blister formation that developed into multiple sites of ulceration, in the present case are most likely due to very high titers of IgG autoantibodies.

In this case, wide-spread blistering and scar formation were characteristic. In other autoimmune blistering skin diseases with autoantibodies directed to proteins at the dermal-epidermal junction, correlations between clinical findings (including scar and milia formation) and the location of the target autoantigen have been made. For example, in bullous pemphigoid, most of the autoantibodies target the juxtamembranous extracellular domain of Col17, the





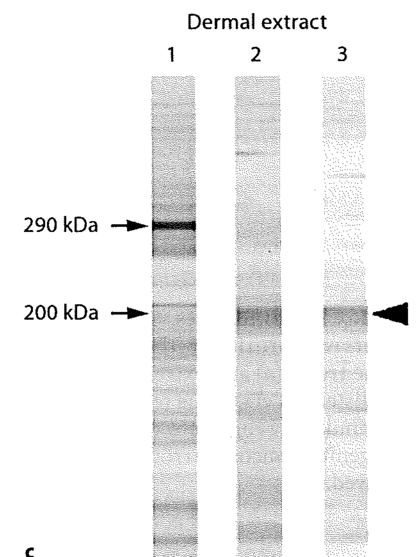
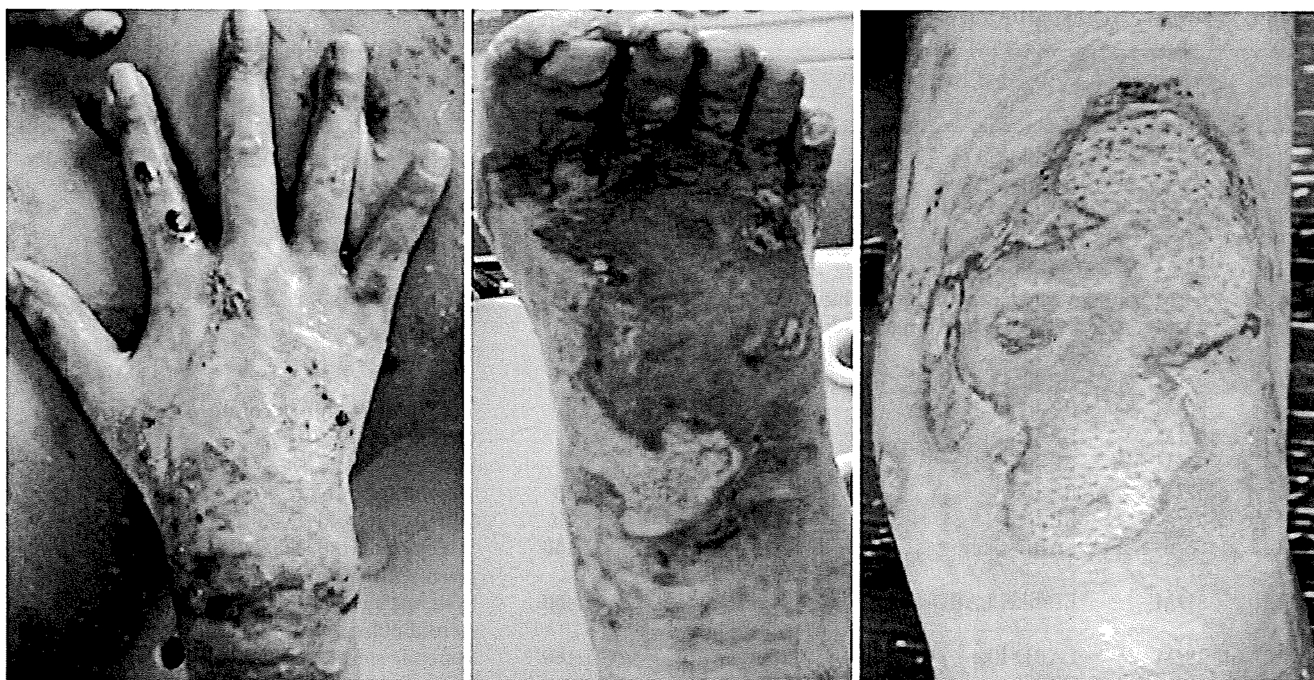


Fig. 2. Histopathology. **a** A subepidermal blister with numerous infiltrating neutrophils (arrows) and eosinophils (arrowheads) was observed. **b** A direct immunofluorescence study demonstrated linear in vivo deposition of IgG and C3 along the epidermal basement membrane. An indirect immunofluorescence study using 1 M NaCl-split human skin as a substrate revealed that most autoantibodies reacted to the dermal side (arrows) as well as some weak staining on the epidermal side of the artificial split (arrowheads). DIF = Direct immunofluorescence; IIF = indirect immunofluorescence. **c** Immunoblotting analysis with a patient's serum using normal human dermal extracts. Control epidermolysis bullosa acquisita serum reacted with the 290-kDa epidermolysis bullosa acquisita antigen (lane 1). Control serum from an anti-p200 patient (lane 2) and that from the present case (lane 3) reacted with the 200-kDa anti-p200 pemphigoid antigen (arrowhead).

NC16A domain and intracytoplasmic BP230. Both of them are present in the upper lamina lucida, and they seldom result in scar formation [22]. In contrast, both mucous membrane pemphigoid and epidermolysis bullosa acquisita are likely to result in scar or milia formation [22–24]. Autoantibodies in mucous membrane pemphigoid and epidermolysis bullosa acquisita patients are directed against the C terminus of Col17 or laminin 5, and type VII collagen, respectively. All of these autoantigens are present along the lamina densa [25], or comprise the anchoring fibrils under it [23]. In anti-p200 pemphigoid, autoantibodies



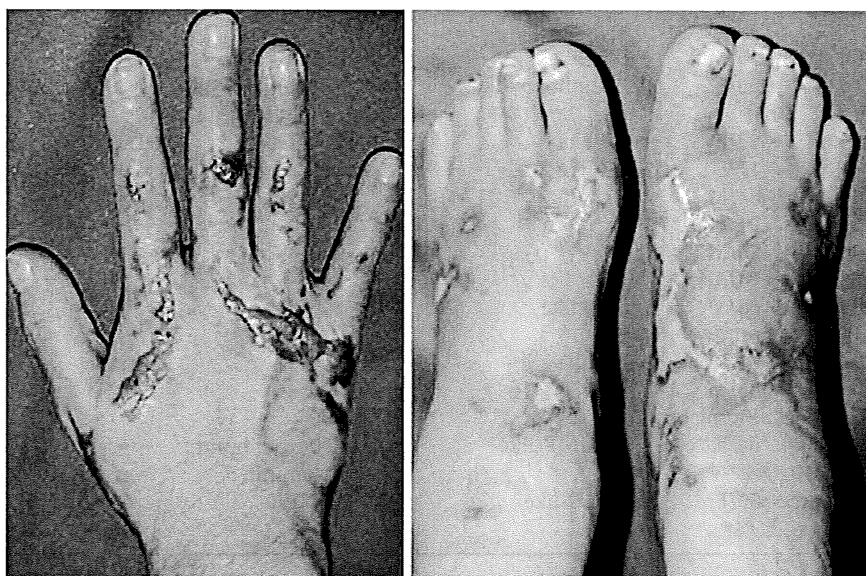


Fig. 3. Clinical features, 14 days after initial treatment. Note the deep ulcers on the hand, foot and knee, which all resulted from blisters (**a**). Thirty days after methylprednisolone pulse therapy, scar formation was evident on the hands and feet (**b**).

from patients react with the lower lamina lucida [1–4]. Therefore, relatively deep presentation of anti-p200 pemphigoid autoantigen might explain the ulcer formation, which was similar to that observed in membrane pemphigoid and epidermolysis bullosa acquisita. In fact, 5 cases of anti-p200 pemphigoid with scar formation have been reported so far (table 1).

Most of the previous cases of anti-p200 pemphigoid have been successfully treated with mild-to-moderate immunosuppressive therapies [1, 6, 8, 9], while azathioprine [3, 18] and intravenous immunoglobulin [13, 26, 27] have also been used in some cases (table 1). In these severe anti-p200 pemphigoid cases that are refractory to oral prednisolone treatment, the most effective alternative treatments are still being investigated. In our present case, methylprednisolone pulse therapy was effective enough to reduce disease activity. So far, only 2 other cases have been reported in which methylprednisolone pulse therapy was effective for the treatment of anti-p200 pemphigoid [13, 28]. Our present case indicates that methylprednisolone pulse

Table 1. Reported cases of anti-p200 pemphigoid in the English-language literature

Patient No.	Age, years/sex	Clinical features	Detected antigen	IIF titer	Treatment	Scar formation
1 [1]	72/F	BP-like	200/230	1:160	Cyclosporine	–
2 [2]	54/M	BP-like	200	1:80	TC, colchicine	–
3 [17]	31/F	BP-like	200/210	1:10	Topical steroid	–
4 [8]	64/M	DH-like	180/200		Systemic steroid	–
5 [10]	72/M	LABD-like	200		DDS	–
6 [16]	64/M	Vesicular pemphigoid-like	200		Systemic steroid, DDS	–
7 [16]	64/M	Vesicular pemphigoid-like	200		Systemic steroid, DDS	–
8 [7]	81/M	BP-like	200		TC, NA	–
9 [14]	69/M	BP-like	200/230		MINO	–
10 [14]	70/M	BP-like	200		TC, NA, systemic steroid	–
11 [14]	54/M	BP-like	200		Cyclosporine	–
12 [14]	76/M	BP-like	200/230		Systemic steroid	–
13 [5]	52/M	BP-like	200	1:40	Topical steroid	–
14 [6]	61/M	BP-like	200	1:40	Systemic steroid, DDS	–
15 [11]	66/F	LABD-like	200	1:160	Systemic steroid, TC, DDS, plasmapheresis	–
16 [13]	51/M	BP-like, LABD-like	200/260	1:400	Systemic steroid, IVIG, DDS, steroid pulse mycophenolate mofetil	–
17 [12]	28/M	LABD-like	200	1:160	Systemic steroid, azathioprine	+
18 [35]	49/M	BP-like	200		Systemic steroid, DDS	–
19 [28]	29/F	Vesicular pemphigoid-like	200/290	1:160	Systemic steroid, MINO, NA, steroid pulse	–
20 [9]	75/M	DH-like	200		Systemic steroid, cyclosporine	–
21 [3]	56/F	Pompholyx-like	165/200 (α_3-subunit of laminin 5)	1:80	Systemic steroid, azathioprine	–
22 [29]	52/F	BP-like	200/290	1:32	Systemic steroid	+
23 [15]	17/F	BP-like	200	1:320	Systemic steroid, DDS	+
24 [36]	84/M		200			–
25 [36]	91/F		200			–
26 [36]	50/M		200			–
27 [36]	74/M		200			–
28 [26]	65/M	EBA-like	200/290		Systemic steroid, DDS, cyclosporine, mycophenolate mofetil, IVIG	+
29 [18]	61/M	BP-like	200	1:20	Systemic steroid, DDS	–
30 [18]	45/M		200		Topical steroid	–
31 [18]	Young boy		200			
32 [27]	64/F	ALMMP-like	200/γ_2-subunit of laminin 5		Steroid, MINO, IVIG	+
Present case	53/F	LABD-like	180/200	>640-fold dilution	Systemic steroid, MINO, NA, steroid pulse	+

BP = Bullous pemphigoid; DH = dermatitis herpetiformis; LABD = linear IgA bullous dermatosis; EBA = epidermolysis bullosa acquisita; ALMMP = antilaminin 5 mucous membrane pemphigoid; IIF = indirect immunofluorescence; TC = tetracycline; DDS = dapsone; NA = nicotinamide; MINO = minocycline; IVIG = intravenous immunoglobulin.

therapy might, therefore, be effective for severe and refractory anti-p200 pemphigoid cases.

Interestingly, this case also had IgG autoantibodies directed against Col17 present. Coexistence of autoantibodies other than anti-p200 autoantigen in anti-p200 pemphigoid is uncommon, but cases with autoantibodies to the α_3-chain of laminin 5 [3], the γ_2-subunit of laminin 5 [27] and type VII collagen [28, 29] have been reported (table 1). There has been only 1 case reported previously in which autoantibodies to both Col17 and p200 autoantigen were detected (table 1) [8]. In autoimmune diseases, it is well known that autoantibodies of patients in the later disease stages are more likely to react with multiple sites of autoantigens than those of patients in early stages of the disease. This is known as 'epitope spreading' [30–32]. However, in

our case, IgG autoantibodies against Col17 could already be detected from the very early stages when the patient initially presented at our clinic. IgG autoantibodies in bullous pemphigoid patients have been reported to be directly pathogenic as they are able to induce separation of the dermis and epidermis in vitro [33]. In addition, IgG autoantibodies directed against the NC16A domain in Col17 from bullous pemphigoid patients have recently been reported to be able to induce detachment of the dermis and epidermis in vivo in a Col17-humanized mouse model [34]. Taken together, we have to assume that autoantibodies to Col17 in the present case, such as those in bullous pemphigoid patients, might also contribute to blister formation, and may also be related to the severity seen in this case.

Acknowledgements

We thank Dr. James R. McMillan and Heather Ann Long for their kind help with English-language editing and proofreading.

References

1 Chen KR, Shimizu S, Miyakawa S, Ishiko A, Shimizu H, Hashimoto T: Coexistence of psoriasis and an unusual IgG-mediated subepidermal bullous dermatosis: identification of a novel 200-kDa lower lamina lucida target antigen. Br J Dermatol 1996;134:340–346.
2 Zillikens D, Kawahara Y, Ishiko A, Shimizu H, Mayer J, Rank CV, Liu Z, Giudice GJ, Tran HH, Marinkovich MP, Brocker EB, Hashimoto T: A novel subepidermal blistering disease with autoantibodies to a 200-kDa antigen of the basement membrane zone. J Invest Dermatol 1996;106:465–470.
3 Shimanovich I, Petersen EE, Weyers W, Sitaru C, Zillikens D: Subepidermal blistering disease with autoantibodies to both the p200 autoantigen and the α3 chain of laminin 5. J Am Acad Dermatol 2005;52:S90–S92.
4 Amrei D, Christian R, Takashi H, Zillikens D, Shimanovich I: Anti-p200 pemphigoid: a novel autoimmune subepidermal blistering disease. J Dermatol 2007;34:1–8.
5 Zillikens D, Ishiko A, Jonkman MF, Chimanovitch I, Shimizu H, Hashimoto T, Brocker EB: Autoantibodies in anti-p200 pemphigoid stain skin lacking laminin 5 and type VII collagen. Br J Dermatol 2000;143:1043–1049.
6 Egan CA, Yee C, Zillikens D, Yancey KB: Anti-p200 pemphigoid: diagnosis and treatment of a case presenting as an inflammatory subepidermal blistering disease. J Am Acad Dermatol 2002;46:786–789.
7 Mascaro JM Jr, Zillikens D, Giudice GJ, Caux F, Fleming MG, Katz HM, Diaz LA: A subepidermal bullous eruption associated with IgG autoantibodies to a 200 kD dermal antigen: the first case report from the United States. J Am Acad Dermatol 2000;42:309–315.
8 Salmhofer W, Kawahara Y, Soyer HP, Kerl H, Nishikawa T, Hashimoto T: A subepidermal blistering disease with histopathological features of dermatitis herpetiformis and immunofluorescence characteristics of bullous pemphigoid: a novel subepidermal blistering disease or a variant of bullous pemphigoid? Br J Dermatol 1997;137:599–604.
9 Yasuda H, Tomita Y, Shibaki A, Hashimoto T: Two cases of subepidermal blistering disease with anti-p200 or 180-kD bullous pemphigoid antigen associated with psoriasis. Dermatology 2004;209:149–155.
10 Kawahara Y, Matsuo Y, Hashimoto T, Nishikawa T: A case of unique subepidermal blistering disease with autoantibodies against a novel dermal 200-kD antigen. Dermatology 1998;139:738–759.
11 Watanabe M, Tsunoda T, Tagami H: A subepidermal blistering dermatosis associated with coexistent IgG and IgA anti-dermal basement membrane zone antibodies: demonstration of IgG antibodies reactive against a 200-kDa dermal antigen. Eur J Dermatol 2002;12:603–606.
12 Umemoto N, Demitsu T, Toda S, Noguchi T, Suzuki SI, Kakurai M, Yamada T, Suzuki M, Nakagawa H, Komai A, Hashimoto T: A case of anti-p200 pemphigoid clinically mimicking inflammatory epidermolysis bullosa acquisita. Br J Dermatol 2003;148:1058–1059.
13 Morris SD, Mallipeddi R, Oyama N, Gratian MJ, Harman KE, Bhogal BS, Black MM, Eady RA, Hashimoto T, McGrath JA: Psoriasis bullosa acquisita. Clin Exp Dermatol 2002;27:665–669.
14 Kawahara Y, Zillikens D, Yancy KB, Marinkovich MP, Nie Z, Hashimoto T, Nishikawa T: Subepidermal blistering disease with autoantibodies against a novel dermal 200-kD antigen. J Dermatol Sci 2000;23:93–102.
15 Yamane N, Sawamura D, Nishie W, Abe M, Kodama K, Adachi K, Nakamura H, Ishii N, Hashimoto T, Shimizu H: Anti-p200 pemphigoid in a 17-year-old girl successfully treated with systemic corticosteroid and dapsone. Br J Dermatol 2007;156:1075–1078.
16 Inoh Y, Nishikawa T, Hashimoto T: The vesicular pemphigoid phenotype may be related to antibodies to a 200-kDa antigen in the lower lamina lucida. Br J Dermatol 1998;139:738–759.
17 Saeki H, Hayashi N, Komine N, Soma Y, Shimada S, Watanabe K, Hasimoto T: A case of generalized pustular psoriasis followed by bullous disease: an atypical case of bullous pemphigoid or a novel bullous disease? Br J Dermatol 1996;134:152–155.
18 Hofmann SC, Voith U, Sasaki T, Trueb RM, Nischt R, Bruckner-Tuderman L: The autoantigen in anti-p200 pemphigoid is synthesized by keratinocytes and fibroblasts and is distinct from nidogen-2. J Invest Dermatol 2008;128:87–95.
19 Tsuji Y, Kawashima T, Yokota K, Tateishi Y, Tomita Y, Matsumura T, Shimizu H: Clinical and serological transition from pemphigus vulgaris to pemphigus foliaceus demonstrated by desmoglein ELISA system. Arch Dermatol 2002;138:95–96.
20 Tsuji-Abe Y, Akiyama M, Yamanaka Y, Kikuchi T, Sato-Matsumura KC, Shimizu H: Correlation of clinical severity and ELISA indices for the NC16A domain of BP180 measured using BP180 ELISA kit in bullous pemphigoid. J Dermatol Sci 2005;37:145–149.
21 Schmidt E, Obe K, Brocker EB, Zillikens D: Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. Arch Dermatol 2000; 136:174–178.
22 Wojnarowska F, Venning VA, Burge SM: Immunobullous diseases; in Burns T, Breathnach S, Cox N, Griffiths C (eds): Rook's Textbook of Dermatology, ed 7. Oxford, Blackwell, 2004, vol 2, pp 41.1–41.59.
23 Fleming TE, Korman NJ: Cicatricial pemphigoid. J Am Acad Dermatol 2000;43:571–591.
24 Engineer L, Ahmed AR: Emerging treatment for epidermolysis bullosa acquisita. J Am Acad Dermatol 2001;44:818–828.
25 Bedane C, McMillan JR, Balsding SD, Bernard P, Prost C, Bonnetblanc JM, Diaz LA, Eady RA, Giudice GJ: Bullous pemphigoid and cicatricial pemphigoid autoantibodies react with ultrastructurally separable epitopes on the BP180 ectodomain: evidence that BP180 spans the lamina lucida. J Invest Dermatol 1997;108:901–907.

26 Pastar Z, Rados J, Lipozencic J, Dobric I, Marinovic B, Ishii N, Hashimoto T: Case of concurrent epidermolysis bullosa acquisita and anti-p200 pemphigoid: how to treat it? Int J Dermatol 2007;46:295–298.
27 Mitsuya J, Hara H, Ito K, Ishii N, Hashimoto T, Terui T: Metastatic ovarian carcinoma-associated subepidermal blistering disease with autoantibodies to both the p200 dermal antigen and the gamma 2 subunit of laminin 5 showing unusual clinical features. Br J Dermatol 2008;158:1354–1357.
28 Furukawa H, Miura T, Takahashi M, Nakamura K, Kaneko F, Ishii F, Komai R, Hashimoto T: A case of anti-p200 pemphigoid with autoantibodies against both a novel 200-kD dermal antigen and the 290-kD epidermolysis bullosa acquisita antigen. Dermatology 2004;209:145–148.
29 Yamada T, Suzuki M, Koike Y, Kida K, Murata S, Hashimoto T, Ohtsuki M: A case of epidermolysis bullosa acquisita with autoantibody to anti-p200 pemphigoid antigen and exfoliative esophagitis. Dermatology 2006; 212:381–384.
30 Fairley JA, Woodley DT, Chen M, Giudice GJ, Lin MS: A patient with both bullous pemphigoid and epidermolysis bullosa acquisita: an example of intermolecular epitope spreading. J Am Acad Dermatol 2004;51:118–122.
31 Maeda JY, Moura AK, Maruta CW, Santi CG, Prisayanh PS, Aoki V: Changes in the autoimmune blistering response: a clinical and immunopathological shift from pemphigus foliaceus to bullous pemphigoid. Clin Exp Dermatol 2006;31:653–655.
32 Peterson JD, Chang AJ, Chan LS: Clinical evidence of an intermolecular epitope spreading in a patient with pemphigus foliaceus converting into bullous pemphigoid. Arch Dermatol 2007;143:272–274.
33 Sitaru C, Schmidt E, Petermann S, Munteanu LS, Brocker EB, Zillikens D: Autoantibodies to bullous pemphigoid antigen 180 induce dermal-epidermal separation in cryosections of human skin. J Invest Dermatol 2002;118:664–671.
34 Nishie W, Sawamura D, Goto M, Ito K, Shibaki A, McMillan JR, Sakai K, Nakamura H, Olasz E, Yancey KB, Akiyama M, Shimizu H: Humanization of autoantigen. Nat Med 2007;13:378–383.
35 Cho SB, Kim SC: A Korean case of anti-p200 pemphigoid. Yonsei Med J 2003;44:931–934.
36 Rose C, Weyers W, Denisjuk N, Hillen U, Zillikens D, Shimanovich I: Histopathology of anti-p200 pemphigoid. Am J Dermatopathol 2007;29:119–124.

Immunological Reconstitution after Autologous Hematopoietic Stem Cell Transplantation in Patients with Systemic Sclerosis: Relationship Between Clinical Benefits and Intensity of Immunosuppression

TOSHIYUKI BOHGAKI, TATSUYA ATSUMI, MIYUKI BOHGAKI, AKIRA FURUSAKI, MAKOTO KONDO, KAZUKO C. SATO-MATSUMURA, RIICHIRO ABE, HIROSHI KATAOKA, TETSUYA HORITA, SHINSUKE YASUDA, YOSHIHARU AMASAKI, MITSUFUMI NISHIO, KEN-ICHI SAWADA, HIROSHI SHIMIZU, and TAKAO KOIKE

ABSTRACT. ***Objective.*** To analyze the relationship between clinical benefits and immunological changes in patients with systemic sclerosis (SSc) treated with autologous hematopoietic stem cell transplantation (HSCT).

Methods. Ten patients with SSc were treated with high-dose cyclophosphamide followed by highly purified CD34+ cells (n = 5) or unpurified grafts (n = 5). Two groups of patients were retrospectively constituted based on their clinical response (good responders, n = 7; and poor responders, n = 3). As well as clinical findings, immunological reconstitution through autologous HSCT was assessed by fluorescence-activated cell sorter analysis, quantification of signal joint T cell receptor rearrangement excision circles (sjTREC), reflecting the thymic function, and *foxp3*, a key gene of regulatory T cells, mRNA levels.

Results. Patients' clinical and immunological findings were similar between good and poor responders, or CD34-purified and unpurified groups at inclusion. The sjTREC values were significantly suppressed at 3 months after autologous HSCT in good responders compared with poor responders (p = 0.0152). Reconstitution of CD4+CD45RO- naive T cells was delayed in good responders compared with poor responders. The phenotype of other lymphocytes, cytokine production in T cells, and *foxp3* gene expression levels after autologous HSCT did not correlate with clinical response in good or poor responders. Clinical and immunological findings after autologous HSCT were similar between CD34-purified and unpurified groups.

Conclusion. Our results suggest that immunosuppression intensity, sufficient to induce transient suppression of thymic function, is attributable to the feasible clinical response in patients with SSc treated with autologous HSCT. Appropriate monitoring of sjTREC values may predict clinical benefits in transplanted SSc patients after autologous HSCT. (First Release May 15 2009; J Rheumatol 2009;36:1240–8; doi:10.3899/jrheum.081025)

Key Indexing Terms:
SYSTEMIC SCLEROSIS
HEMATOPOIETIC STEM CELL TRANSPLANTATION
IMMUNOLOGICAL RECONSTITUTION

From the Department of Medicine II and the Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo; and Third Department of Internal Medicine, Akita University School of Medicine, Akita, Japan.

Supported by a grant from the Japanese Ministry of Health, Labor and Welfare.

T. Bohgaki, MD, PhD; T. Atsumi, MD, PhD; M. Bohgaki, MD, PhD; A. Furusaki, MD, PhD; M. Kondo, MD, PhD, Department of Medicine II; K.C. Sato-Matsumura, MD, PhD; R. Abe, MD, PhD, Department of Dermatology; H. Kataoka, MD, PhD; T. Horita, MD, PhD; S. Yasuda, MD, PhD; Y. Amasaki, MD, PhD; M. Nishio, MD, PhD, Department of Medicine II, Hokkaido University Graduate School of Medicine; K. Sawada, MD, PhD, Third Department of Internal Medicine, Akita University School of Medicine; H. Shimizu, MD, PhD, Department of Dermatology; T. Koike, MD, PhD, Department of Medicine II, Hokkaido University School of Medicine.

Address reprint requests to Dr. T. Atsumi, Department of Medicine II, Hokkaido University Graduate School of Medicine, N15, W7, Kita-ku, Sapporo 060-8638, Japan. E-mail: at3tat@med.hokudai.ac.jp

Accepted for publication January 8, 2009.

Systemic sclerosis (SSc) is an autoimmune disease characterized by the presence of skin sclerosis, organ fibrosis, and autoantibodies[1]. Despite extensive research on autoimmunology and endotheliology, its pathophysiology has been far from conclusive[2,3]. The skin and organ manifestations of SSc are, in general, slowly progressive and chronically disabling. In some patients, however, they can be rapidly progressive and fatal due to organ involvements such as interstitial pneumonia, arrhythmia, and renal failure. Severe organ involvement frequently occurs within the first 3 years of disease[1]. These clinical features affect daily living activity and life expectancy in patients with SSc.

Autologous hematopoietic stem cell transplantation (HSCT) has been indicated for patients with autoimmune diseases, resulting in great success particularly in patients with SSc[4-10]. Autologous HSCT is one of the treatments in

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2009. All rights reserved.

patients suffering from hematological malignant diseases. For the practice of autologous HSCT against those malignant diseases, graft manipulation using antibody specific for CD34, a marker of human hematopoietic stem cells, is usually essential to deplete malignant cells from the graft. On the other hand, patients treated with CD34+-selected autologous HSCT (CD34-HSCT) may have infectious complications during hematological recovery more frequently than patients treated with unselected autologous HSCT (unselected-HSCT)[11]. The graft manipulation was performed in many patients with severe autoimmune diseases treated by autologous HSCT in consideration of depleting autoreactive lymphocytes and inducing profound clinical remission. Meanwhile, it has been debated whether CD34+ cell selection in the graft is necessary or not[9,12].

The difference in conditioning regimens is not related to the clinical benefits, and about one-third of transplanted patients do not benefit from these intensive immunosuppressive treatment[9,13]. Clinical response may depend on profound qualitative immunological changes obtained by autologous HSCT in patients with systemic lupus erythematosus or multiple sclerosis[14,15]. Little is known as to why and how patients with SSc have clinical benefits of autologous HSCT. The aim of our study was to elucidate the relationship between clinical effect and alteration of immunological profiles in patients with SSc treated with autologous HSCT.

MATERIALS AND METHODS

Patients. Our study was approved by the ethical committee of Hokkaido University and written informed consent was obtained from all participants. Thirty-one patients with SSc, all of whom met the American College of Rheumatology preliminary criteria[16], were screened for our study. All patients developing SSc within the last 3 years onset fulfilled at least 1 of the following: early rapidly progressive diffuse skin sclerosis despite continuing treatment, refractory skin ulcers, interstitial lung disease confirmed by lung computed tomography (CT), reversible cardiac involvement such as arrhythmia and cardiomegaly, renal involvement with hypertension, persistent urinalysis abnormalities, and microangiopathic hemolytic anemia. Patients were excluded from the study when they were over 60 years old, or had uncontrolled arrhythmia, left ventricular ejection fraction on echocardiography below 45%, carbon dioxide diffusion lung capacity (DLCO) below 45% predicted, serum creatinine above 176.8 µmol/L (2.0 mg/dl) and glomerular filtration rate (GFR) below 40 ml/min/m^2. All enrolled patients were evaluated clinically at the time of diagnosis and on regular visits for followup.

Thirty-five healthy controls were also enrolled in the study.

Transplantation procedure and followup. The mobilization regimen comprised recombinant human granulocyte colony-stimulating factor (rhG-CSF) and intravenous cyclophosphamide (4 g/m^2). In 5 patients treated with CD34-HSCT, enriched CD34+ graft, prepared using CliniMACS® system (Miltenyi Biotec, Germany) was stored in liquid nitrate until use for transplant. Graft manipulation was not performed in the next 5 patients treated with unselected-HSCT.

We treated all SSc patients with intravenous cyclophosphamide (200 mg/kg, divided into 4 days) followed by autologous HSCT. rhG-CSF was administered from the second day of transplantation of frozen-thawed autologous enriched CD34+ grafts or frozen-thawed autologous unselected grafts. T cell depleting antibodies such as antithymocyte globulin, antilymphocyte globulin and anti-CD52 antibodies (Campath) were not administered in our patients.

We assessed the improvement of skin sclerosis by the modified Rodnan total thickness skin score (mRTSS). Electrocardiogram and echocardiography were used to evaluate the cardiac function, chest radiograph, chest high resolution CT, and spirometry to evaluate pulmonary function, renogram to evaluate renal function, and serological tests to assess other organ involvement and the presence of autoantibodies.

Lymphocyte phenotyping. Peripheral blood mononuclear cells (PBMC) were prepared from heparinized venous blood by Ficoll-Paque Plus® (Amersham Biosciences Corp., NJ, USA).

We assessed the subpopulation of peripheral lymphocytes by immunofluorescence staining of PBMC with anti-human CD3-Cy-Chrome, CD4-fluorescein isothiocyanate (FITC), CD8-FITC, CD19-FITC, TCRγδ-FITC, CD3-phycoerythrin (PE), CD8-PE, CD45RO-PE, CD25-PE, HLA-DR-PE, and CD69-PE (BD Biosciences Pharmingen, San Diego, CA).

The expression levels of interferon (IFN)-γ and interleukin (IL)-4 were studied in the cytoplasm of peripheral CD4+ or CD8+ T cells. Briefly, we stimulated PBMC with phorbol myristate acetate (50 ng/ml) and ionomycin (250 ng/ml) for 6 h in RPMI 1640 containing 10% heat-inactivated fetal bovine serum and monensin (2 µM) at 37°C in 5% carbon dioxide. We evaluated the IFN-γ or IL-4 expression on T cells by staining with anti-CD3-Cy-Chrome, anti-CD8-FITC and -PE, anti-IFN-γ-FITC, and anti-IL-4-PE using Cytofix/Cytoperm Plus® (BD Biosciences Pharmingen) according to the manufacturer's instructions. Immunostained cells were analyzed using a FACSCalibur™ flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Quantification of thymic signal joint T cell receptor rearrangement excision circles (sjTREC). Thymic sjTREC on genomic DNA from PBMC was quantified by real-time quantitative polymerase chain reaction (PCR) (ABI PRISM® 7000; Applied Biosystems, Foster City, CA) according to the method of Douek, *et al*[17]. The sjTREC values were corrected by the percentage of CD3+ cells in the sample and were then expressed as numbers of sjTREC/µg of CD3+ cells DNA according to the method of Farge, *et al*[18]. Values were measured before autologous HSCT, then at 3, 6, and 12 months after autologous HSCT.

Quantification of foxp3 *gene expression levels.* Total RNA were isolated from PBMC using TRIzol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA (1 µg) was reverse transcribed by ReverTraAce (Toyobo, Osaka, Japan), in the presence of oligo(dT)12-18 primers (Invitrogen) according to the manufacturer's instructions. We performed real-time PCR using the ABI PRISM® 7000 Sequence Detection System and specific primers for *foxp3* and *gapdh* from TaqMan® Gene Expression Assays (Applied Biosystems).

Statistical analysis. We used the Mann-Whitney U-test to analyze the difference among each value otherwise indicated. The changes in mRTSS and phenotype of lymphocytes after the autologous HSCT were compared with values at inclusion using the Wilcoxon signed rank test. Female-male ratio in each group was assessed using Fisher's exact probability test. The sjTREC values in healthy individuals were assessed using the Spearman's correlation test. Calculations were performed using the statistical software package JMP version 5.0 (SAS Institute Inc., Cary, NC). P values less than 0.05 were considered significant.

RESULTS

Between November 2000 and July 2006, 11 consecutive patients meeting the criteria in our study were enrolled and 10 patients were transplanted out of 31 screened patients with SSc for autologous HSCT treatment. One patient was not transplanted because of her mobilization failure. First 5 patients were treated with CD34-HSCT. Subsequent 5 patients were treated with unselected-HSCT. The character-

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2009. All rights reserved.

istics of patients treated with autologous HSCT are shown in Table 1. Mean age at inclusion, mean mRTSS before mobilization and mean durations from SSc onset to the treatment were similar between patients treated with CD34-HSCT and unselected-HSCT. Several treatments such as D-penicillamine, prostaglandin derivatives, and corticosteroids were not feasible for our patients. All patients were followed up until July 2007 (40.7 ± 25.6 mos).

Mean number of infused CD34+ cells was not different between CD34-HSCT and unselected-HSCT groups. Mean time needed to achieve a neutrophil count greater than $0.5 \times 10^9/l$ and a platelet count greater than $50 \times 10^9/l$ were not different between 2 groups. Cytomegalovirus antigenemia were shown in 3 patients out of all transplanted patients. Patient 2 had hemophagocytic syndrome on day 6. Patient 3 had adenoviral hemorrhagic cystitis on day 14 and engraftment syndrome on day 15. Patient 7 had engraftment syndrome on day 12. Hemophagocytic syndrome and engraftment syndrome responded to corticosteroid administration. Hemorrhagic cystitis was refractory to acyclovir, vidarabine, ganciclovir, or ribavirin. Patient 3 had the second autologous HSCT using unselected grafts at 3 months after first autologous HSCT using selected CD34+ cells due to recurrent infectious diseases.

Four out of 5 transplanted patients have more than a 25% fall in the skin score compared with baseline values in both groups (Figure 1). Dermal thickness assessed by skin biopsy was also improved in these patients with clinical benefits (data not shown). Additional unselected-HSCT at 3 months after CD34-HSCT did not affect Patient 3's skin manifestation. Cardiac and pulmonary functions were not altered significantly through the treatment in all patients (data not shown). Their serum level of γ-globulin almost remained normal range through autologous HSCT (data not shown). Their serum level of anti-Scl70 antibodies reduced except Patient 2 treated with CD34-HSCT (data not shown). Transplantation related complications during hospitalization are shown in Table 1. There was no significant difference in the incidence of adverse events between both groups and no transplantation related mortality.

We compared immunological reconstitution profile over time between good and poor response groups, and between CD34-HSCT and unselected-HSCT groups. First, we analyzed immunological reconstitution between good and poor response groups. Clinical response to therapy was categorized into major, partial, or no response, or disease progression or relapse according to the method of Farge, *et al*[13]. According to the observed clinical response compared to these criteria, 2 groups of patients were retrospectively constituted: good response group, consisting of 7 patients with sustained major or partial response, and poor response group, consisting of 3 patients (Patient 5, 6, and 7) with no

Table 1. Patients' profile at study inclusion and clinical findings at autologous hematopoietic stem cell transplantation (HSCT).

	Patients Treated with CD34-HSCT					Patients treated with Unselected-HSCT					Mean ± SD		
	1	2	3	4	5	6	7	8	9	10	CD34	Untreated	p
Age, yrs	57	19	54	48	52	43	19	42	30	28	46.0 ± 15.4	32.4 ± 10.1	0.094
Sex, female:male	M	F	F	F	M	M	F	F	F	F	3:2	4:1	1.000
mRTSS, 0–51	38	28	25	15	32	32	17	26	23	20	27.6 ± 8.6	23.6 ± 5.8	0.402
Disease duration, mo	21	31	21	12	36	16	24	18	8	12	24.2 ± 9.4	15.6 ± 6.1	0.141
Interstitial pneumonia	—	—	+	—	+	—	+	—	—	—	—	—	—
GFR, ml/min	76.53	121.43	101.43	114.39	99.32	139.29	120.3	101.8	82.62	103.42	102.6 ± 17.2	109.5 ± 21.3	0.465
DLCO %	83	66.8	52.2	90.9	83.8	92.5	54.7	113.4	48	94.4	75.3 ± 15.7	80.6 ± 28.0	0.465
γ-globulin, %	19.5	24.7	24.1	16.8	12.5	20.5	19.8	—	16.8	16.7	19.5 ± 5.1	18.5 ± 2.0	0.712
Anti-Scl 70, index	< 5	92.3	204.6	8.7	158.6	16.1	128.2	< 5	< 5	202	92.8 ± 90.2	69.3 ± 91.5	0.597
Prior therapies	PG	PG, D, PSL	PG, PSL	PG, PSL	D, PSL	PG	PG	D, PSL	D, PSL	PG	—	—	—
Mobilization	G	G + CYC	G + CYC	G + CYC	G + CYC	G + CYC	G + CYC	G + CYC	G + CYC	G + CYC	—	—	—
Conditioning	CYC	CYC	CYC	CYC	CYC	CYC	CYC	CYC	CYC	CYC	—	—	—
Infused CD34+ cells, $\times 10^6$/kg	2.96	5.21	2.75	3.14	12.7	3.95	2.77	4.28	14.9	2.81	5.4 ± 4.2	5.7 ± 5.2	0.917
Purity, %	96	95	90	93.53	96.59	—	—	—	—	—	94.2 ± 2.6	—	—
Neutrophils > 0.5 $\times 10^9$/l (day)	11	9	11	9	9	8	11	10	10	10	9.8 ± 1.1	9.8 ± 1.1	0.914
Platelets > 50 $\times 10^9$/l (day)	15	21	16	8	11	0	8	11	11	12	14.2 ± 5.0	8.4 ± 4.9	0.138
Transplant related complications	CMV	CMV, HPS	CMV, HC, ES	—	—	—	ES	—	—	—	—	—	—

mRTSS: modified Rodnan total thickness skin score; PG: prostaglandin derivatives; D: d-penicillamine; PSL: prednisolone; G: granulocyte-colony-stimulating factor; CYC: cyclophosphamide; CMV: cytomegalovirus antigenemia; HPS: hemophagocytic syndrome; HC: hemorrhagic cystitis; ES: engraftment syndrome; GFR: glomerular filtration rate; DLCO: diffusion capacity for carbon monoxide.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2009. All rights reserved.