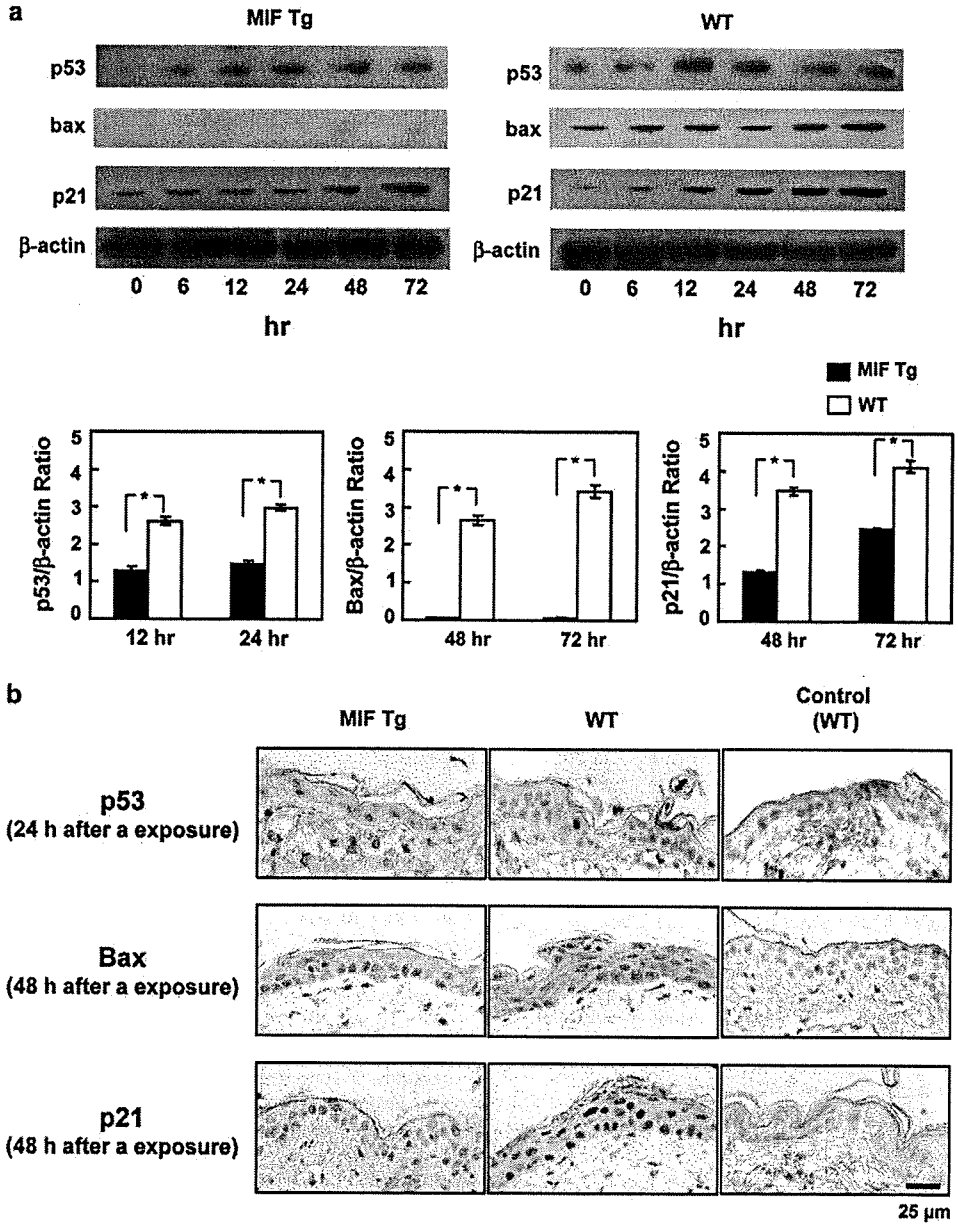

Fig. 5. p53, bax and p21 protein expression in UV-irradiated MIF Tg and WT mice epidermis. (**a**) Western blot analysis of p53, bax and p21 protein expression in unirradiated (0 h) and UVB-irradiated MIF Tg and WT mice skin at various time points. The relative amounts of protein associated with specific antibodies were normalized by the intensities of β-actin ($^{*}P < 0.0001$, $^{**}P < 0.001$, $n = 3$). The data shown are representative of three independent experiments. (**b**) Immunohistochemical analysis for p53, bax and p21 proteins in UVB-irradiated MIF Tg and WT mice skin at 24 h of p53 and at 48 h of bax and p21 immunoreactivity. This experiment was repeated three times with similar results. The scale bar indicates 25 μm.

Discussion

Chronic exposure to solar UV irradiation leads to photoaging, immunosuppression and ultimately carcinogenesis in the skin. Apoptosis and enhanced DNA repair are important p53-mediated responses (32). UVB-induced DNA lesions contribute to cell cycle arrest, DNA repair and finally apoptosis when DNA damage is beyond repair. p53/p21 are responsible for these adaptive protective responses. Moreover, p53 also directly participates in the initiation and regulation of the DNA repair procedure. Therefore, it is extremely important that apoptosis is induced quickly after UV irradiation, without any dysregulation. The current study demonstrated that an earlier onset of carcinogenesis and a higher incidence of tumors were observed in the MIF Tg mice compared with the WT mice after chronic UVB irradiation. In addition, the UVB-induced apoptosis of epidermal keratinocytes was inhibited in the MIF Tg mice. Significantly fewer TUNEL-positive cells were detected in MIF Tg mice in comparison with WT mice. There was a decreased expression of apoptosis-regulatory genes, p53, bax and p21 in MIF Tg mice after UVB irradiation in this study. A previous study has already confirmed that similar protective effects were observed in response to acute UVB light in the MIF Tg mice cornea (33). MIF is upregulated by UVB irradiation in mouse cornea and MIF Tg mice had less apoptotic cells. TUNEL staining in the cornea shows a significantly smaller number of TUNEL-positive nuclei in the MIF Tg mice compared with the WT mice after UV exposure (33).

MIF is a cytokine that not only plays a critical role in several inflammatory conditions but also inhibits p53-dependent apoptotic processes (23,24,34). Hudson *et al.* (23) reported that MIF

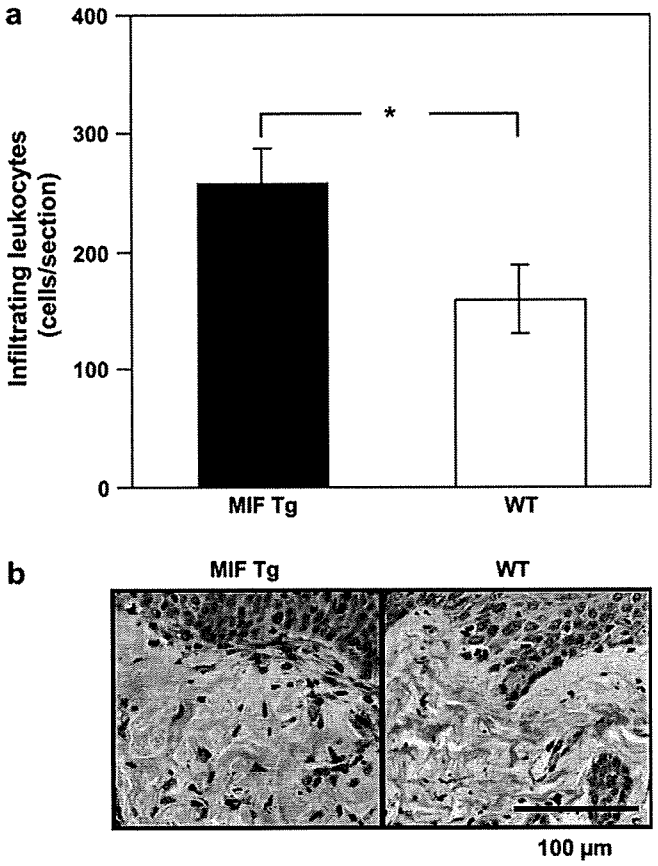

Fig. 6. UVB-induced cutaneous inflammation in MIF Tg and WT mice. (**a**) After three courses of UVB exposure, skin was obtained on day 7 and the paraffin-embedded skin samples (5 μm thick) were processed for routine hematoxylin and eosin staining following a standard protocol. Infiltrating leukocytes (monocyte/macrophages and neutrophils) of the MIF Tg mice were compared with the WT mice. Each value represents the mean ± SEM (n = 5). UVB exposure of MIF Tg mice resulted in greater leukocyte infiltration than that observed in UVB-irradiated WT mice skin (* $P < 0.05$). (**b**) Representative examples of micrographs of hematoxylin and eosin staining are shown from experiments conducted using skin samples (n = 5) that had identical patterns. Bar = 100 μm.

treatment was able to overcome p53 activity and inhibited its transcriptional activity. Recently, Martin *et al.* (35) reported that MIF-deficient mice showed significant increases in p53 activity following acute UVB irradiation, and MIF-deficient mice showed a reduction in tumor incidence in comparison with WT mice following chronic UVB exposure. Previous data from other groups and the current findings suggest that MIF has an inhibitory effect on UVB-induced photodamage by blocking the relevant expression of apoptosis-regulatory genes p53, bax and p21 and MIF plays an important role in UVB-induced tumor development and progression.

The present study also demonstrated that UVB exposure in MIF Tg mice resulted in greater leukocyte infiltration than that of the UVB-irradiated WT mice skin. UVB irradiation enhances the expression of MIF in the epidermis (25) and MIF Tg mice showed higher levels of MIF mRNA expression after UVB exposure in this study. UVB stimulates the production of several proinflammatory cytokines in the skin and these cytokines are known to be involved in the induction of skin carcinogenesis (11,36). For example, TNF-α is the essential cytokine in tumor promotion in mouse skin. Tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate on the skin of TNF-α-deficient mice decreased in comparison with WT mice. Similarly, tumor promotion in IL-6-deficient mice was significantly decreased by 12-*O*-tetradecanoylphorbol-13-acetate compared with the WT mice (11). UVB-induced inflammatory responses, such as the production of cytokines and the infiltration of inflammatory cells, are clearly linked to the development of skin tumors (3,4). The inhibition of this inflammatory response via topical application of an anti-inflammatory drug inhibits the acute inflammatory responses after UVB exposure and decreases tumor formation after chronic exposure (37). MIF has a direct proinflammatory role in inflammatory conditions and tumorigenesis (38). Once released, MIF acts as a proinflammatory cytokine to induce expression of other inflammatory cytokines, including IL-1, IL-6 and TNF-α. Therefore, intense inflammation in MIF Tg mice in response to UVB irradiation was found to correlate with the early onset of carcinogenesis and the higher incidence of tumors after chronic UVB irradiation.

MIF has a wider spectrum of action and exhibits proneoplastic activity. In many tumor cells and pretumor states, increased MIF mRNA can be detected in prostate (39), colon (40) and hepatocellular cancers (41), adenocarcinomas of the lung (42), glioblastomas (43) and melanomas (9). The role of MIF in proneoplastic activity has been examined by several groups. Fingerle *et al.* reported that embryonic fibroblasts from MIF deficient mice exhibit p53-dependent growth alterations, increased p53 transcriptional activity and resistance to ras-mediated transformation (23,24,34). Concurrent deletion of the p53 gene *in vivo* reversed the observed phenotype of cells deficient in MIF. *In vivo* studies showed that fibrosarcomas are smaller in size and have a lower mitotic index in MIF deficient mice relative to their WT counterparts. They concluded direct genetic evidence for a functional link between MIF and the p53 tumor suppressor (23,24,34). The effectiveness of an anti-MIF antibody on reducing tumor growth and neovascularization in lymphoma cells and vascular endothelial cells *in vivo* has been reported (22). Consistent with this finding, anti-MIF antibodies are effective in reducing tumor angiogenesis in melanoma cells (21). This was demonstrated *in vitro* by recombinant MIF in fibroblasts, where growth factor-induced stimulation of these cells resulted in increased MIF concentrations, activation of the ERK-MAP kinase pathway and a subsequent increase in cell proliferation (44). Meyer-Siegler *et al.* (45) has also shown that the addition of TGFβ results in increased MIF expression in a colon cancer cell line. Furthermore, Abe *et al.* (46) observed an increase in cytotoxic T lymphocytes following MIF inhibition as a result of specific antibodies. Moreover, the number of apoptotic tumor cells increased following MIF inhibition. Tumors arising in the MIF knockdown cells grew less rapidly and also showed an increased degree of apoptosis (47). These findings therefore suggest that once keratinocytes are mutated by UVB-induced DNA damage, they may develop into tumor cell, suggested that MIF has a dual role by promoting tumor cell growth and inhibiting the apoptotic processes.

In conclusion, chronic UVB irradiation induces early onset of skin carcinogenesis and the high incidence of tumors in MIF Tg mice. These findings suggest that chronic UVB exposure enhances MIF production, which may inhibit the p53-dependent apoptotic processes, enhance intensive inflammation and thereby induce photocarcinogenesis in the skin. Consequently, this newly identified mechanism may contribute to our overall understanding of photo-induced skin damage, which results in carcinogenesis. These findings are promising for the potential development of MIF inhibitors for therapeutic use and the treatment of photodamaged skin.

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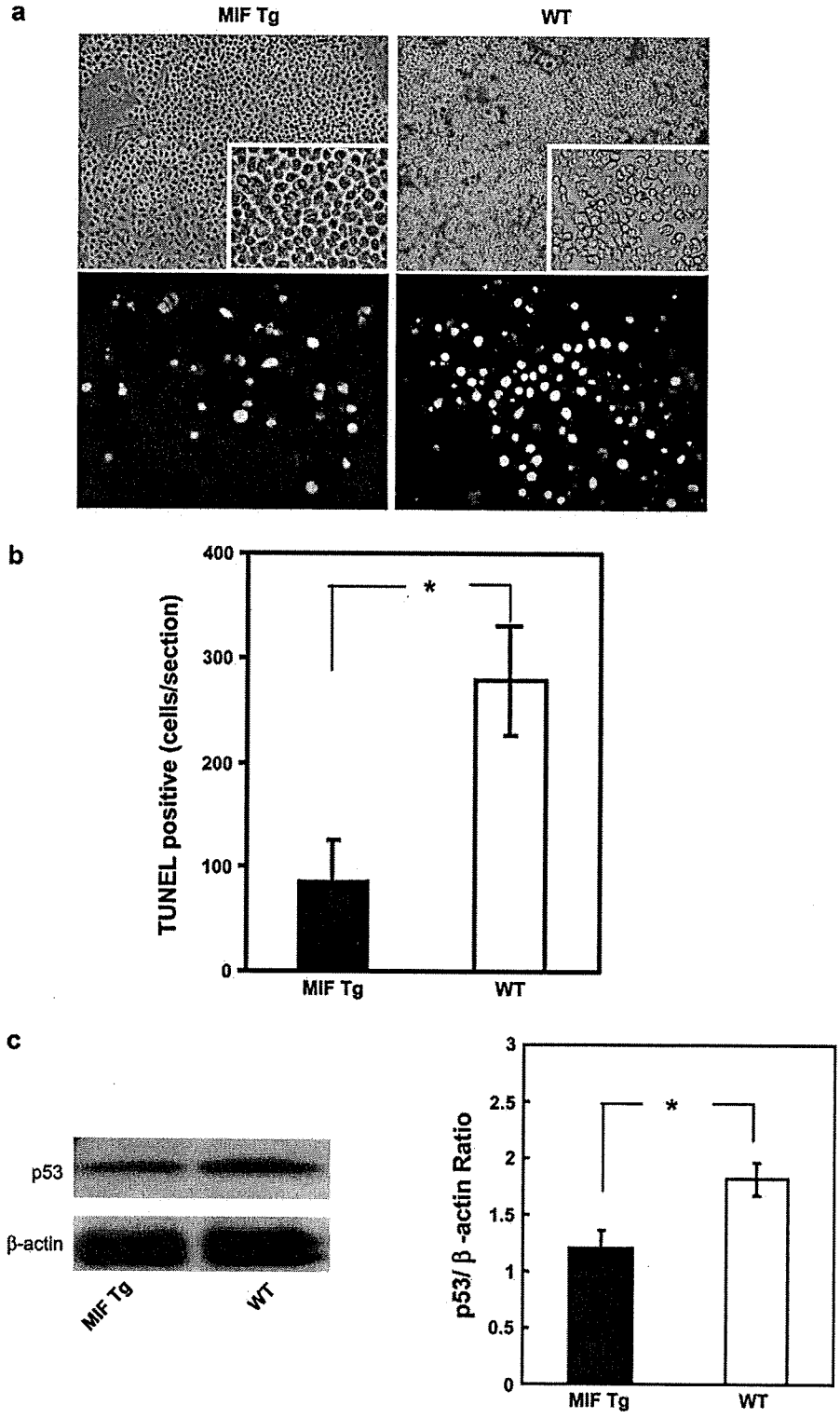

Fig. 7. UVB-induced apoptosis in cultured keratinocytes of MF Tg and WT. (**a**) Cultured keratinocyte from the MIF Tg or WT mice were irradiated with UVB at 50 mJ/cm^2. After 24 h, irradiated cells were analyzed for TUNEL assay. Upper panels indicate morphological pictures. Lower panels indicate TUNEL assay. (**b**) Apoptotic keratinocytes (TUNEL positive) from MIF Tg mice were significantly reduced compared with that of WT mice ($^*P < 0.005$). (**c**) p53 protein expression of UVB-irradiated keratinocytes were analyzed using western blot. The relative amounts of proteins associated with specific antibodies were normalized by the intensities of β-actin. These data shown are representative of three independent experiments. p53 expression of MIF Tg keratinocytes was lower than that of WT mice ($^*P < 0.005$).

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Melanonychia caused by *Stenotrophomonas maltophilia*

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Subungual melanomas often present initially as brown to black nail pigmentation, thus it is important to make a precise diagnosis in cases of melanonychia. Infection or colonization with bacteria or fungi is also known to cause melanonychia.[1] We describe a case of melanonychia caused by *Stenotrophomonas maltophilia* that was suspected to be melanoma.

A 54-year-old man was referred with a 1-year history of an enlarging area of pigmentation in the right great toenail. He was in good health and was taking no medication.

On examination, the great toe nail on his right foot showed partial thickening, onycholysis and irregular pigmentation over most of the nail plate (Fig. 1). Pigmentation of the proximal or lateral nail folds (Hutchinson's sign) was not observed.

Microscopy with potassium hydroxide showed no fungal component in the nail specimen. An excisional biopsy specimen was taken from the pigmented portion of the nail and underlying nail bed showed a large number of Gram-negative bacilli and dark-brown to black pigmentation within the nail plate. The pigmentation in the section disappeared after melanin digestion. Neither melanocyte proliferation nor hypermelanosis was observed in the underlying hyponychium.

Bacterial culture from the nail samples grew *S. maltophilia*, although fungal culture was negative. From these findings, the diagnosis of melanonychia due to *S. maltophilia* was established. After the resection operation, there was no further recurrence of nail pigmentation.

Melanonychia may be due to exogenous substances including bacterial, mycotic and blood pigments, or endogenous melanin pigmentation.[1–3] Bacterial pigmentation, most commonly due to *Pseudomonas aeruginosa* or *Proteus* spp., has a greenish or greyish hue.[2] However, in the present case, the colour was almost black. Melanocytic lesions were excluded by the excisional biopsy of the pigmented nail plate and underlying hyponychium, and the patient was diagnosed with melanonychia due to *S. maltophilia*.

To our knowledge, this is the first case report of melanonychia caused by *S. maltophilia*. *Stenotrophomonas maltophilia* is a nonfermentative Gram-negative bacillus found in various environments such as water, soil, plants and food and in hospitals.[4] *Stenotrophomonas maltophilia* has emerged as an important pathogen capable of causing a broad spectrum of clinical syndromes including pneumonia, bacterial sepsis, urinary tract infections, endocarditis, meningitis and a variety of skin presentations including cellulitis, erythematous nodules, skin ulcers and acral necrosis.[4] *Stenotrophomonas maltophilia* is known to express the tyrosinase gene *mel* and has been shown to

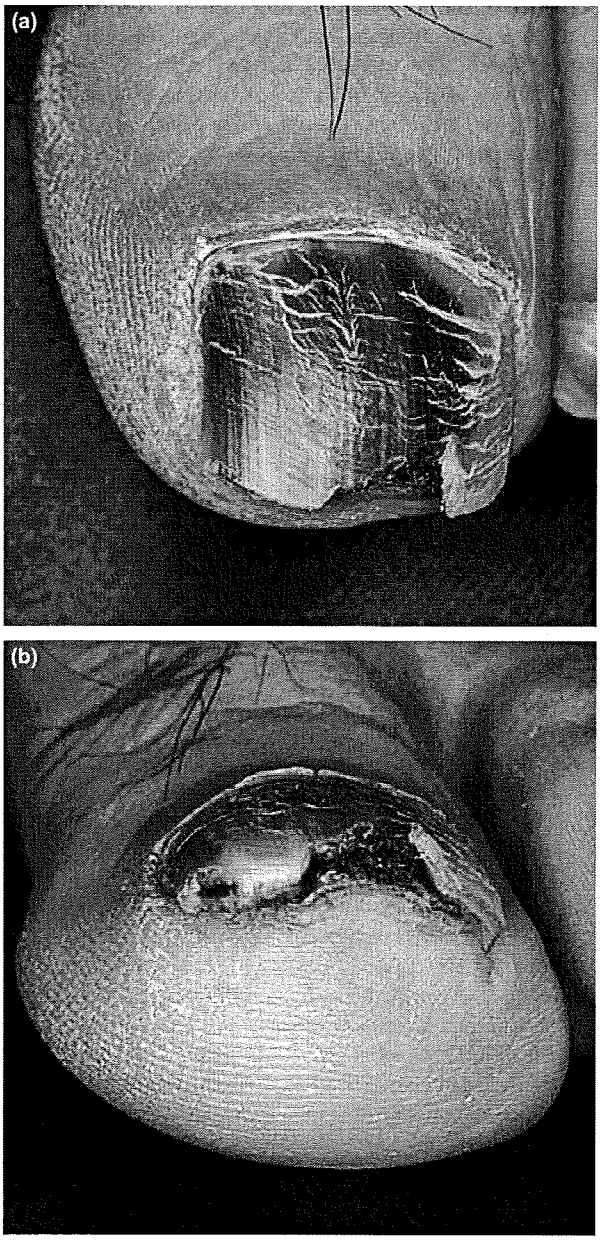

Figure 1 Black discoloration of the first toe nail on the right foot. (a) The thickened, partly onycholytic nail plate with the intact nail fold. The distal portion of the nail had been cut into sections. (b) Pigmentation, mainly in the nail plate but also on the nail bed.

produce melanin.[5] Production of melanin is thought to be linked to protection against environmental insults such as antibiotics.[6] The melanin derived from *S. maltophilia* probably contributed to the pigmentation of the nail plate in this case. Melanonychia caused by *S. maltophilia* may be misdiagnosed as a melanocytic lesion, because a Fontana–Masson stain is positive for nail melanin pigmentation due to *S. maltophilia*, which differs from the fluorescein and pyocyanin pigmentation due to *Pseudomonas aeruginosa*. The present case suggests that non-*Pseudomonas aeruginosa* bacteria such as *S. maltophilia* should be included in the list of causes of a black nail.

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A case of solitary collagenoma localized on the upper lip mimicking mucocele

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Collagenomas are rare hamartomatous malformations of the dermis, characterized by a proliferation of normal collagen tissue. We present a case of isolated (solitary) collagenoma arising in the upper lip, an uncommon location for this disease, and clinically mimicking mucocele.

A 56-year-old Japanese woman was referred to us with a 3-month history of an asymptomatic soft mass on the left upper lip. She was otherwise in good health. Her medical history was not relevant, and there was no family history of a similar disorder.

Physical examination found a slightly raised, soft, elastic, bluish-white nodule on the inner surface of the left upper labial mucosa (Fig. 1). The lesion was well demarcated, and measured 5 mm in diameter with a round shape and smooth surface. No lesions were observed on the trunk, limbs or any other body sites.

Because of the suspicion of mucocele, an excisional skin biopsy was taken for both diagnostic and therapeutic purposes. Histopathological examination found an increased number of collagen bundles in the dermis, which were arranged randomly (Fig. 2a). The collagen bundles stained strongly with Azan–Mallory stain, showing an intense blue colour (Fig. 2b). The lesion had no capsule and was relatively well-defined. Elastic van Gieson stain showed a mild decrease in elastic bundles. Based on the histopathological findings, a diagnosis of solitary collagenoma was made.

Collagenomas, also known as connective tissue naevi of the skin (hamartomas), are composed predominantly of collagen. According to the classification of the genetic inheritance pattern, they are classified as either inherited or acquired.[1,2] The inherited group includes familial cutaneous collagenoma and shagreen patches of tuberous sclerosis, which are inherited in an autosomal dominant matter. The acquired group includes eruptive collagenomas and isolated collagenomas. In both familial and eruptive collagenomas, lesions are characterized clinically by asymptomatic, multiple, skin-coloured papules and nodules distributed symmetrically on the trunk and upper arms.[1,2] In contrast, isolated collagenoma is sporadic, localized to only one body region, and not associated with any disease.

In our patient, the diagnosis of isolated collagenoma, rather than eruptive collagenoma, was made because the lesion was a solitary nodule on the lip, and the patient had no family history of the condition.

The aetiology of collagenomas is still unknown. However, acquired collagenomas occur commonly on trauma-

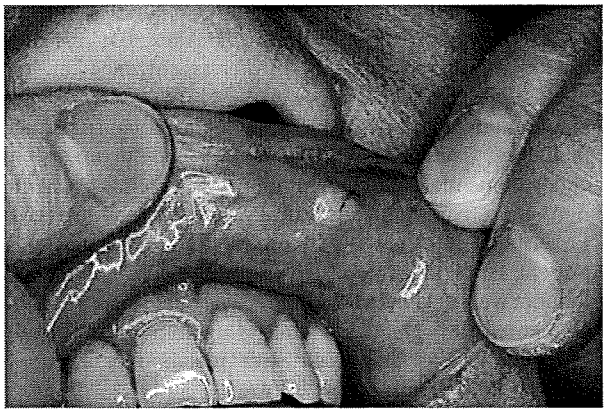

Figure 1 A bluish-white nodule mimicking a mucocele on the inner mucosa of the upper lip.

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

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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

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,

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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

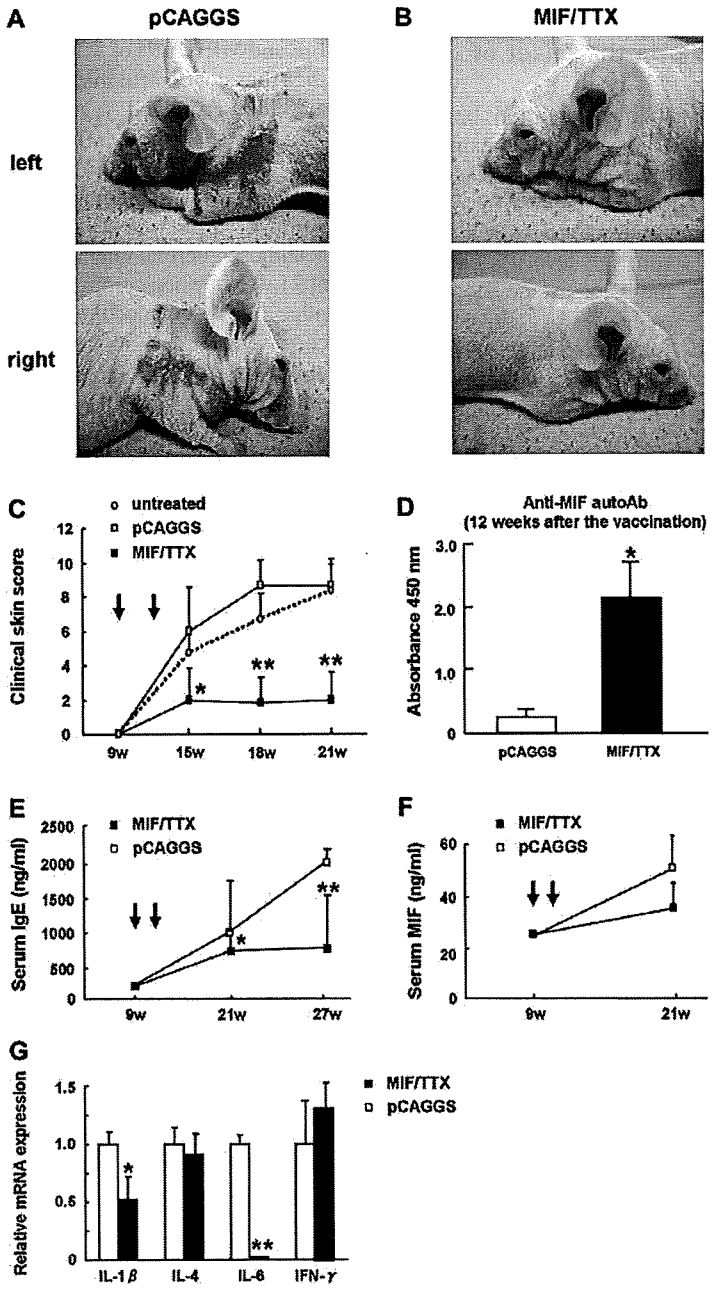

FIG 1. Prevention of the onset of AD by MIF/TTX-DNA vaccine. Nine-week-old DS-Nh mice without skin eruptions were subjected to administration of MIF/TTX or a control plasmid (pCAGGS). Clinical features of 21-week-old DS-Nh mice vaccinated with endotoxin-free pCAGGS **(A)** and MIF/TTX (**B**; 12 weeks 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

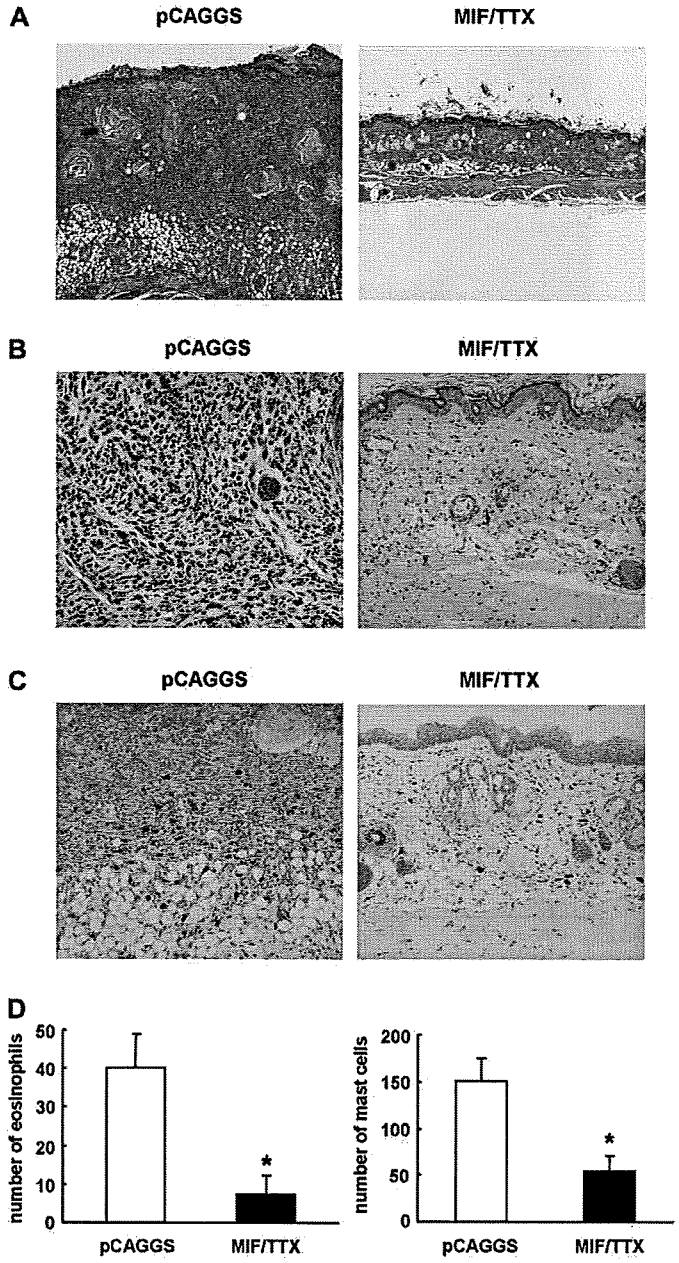

FIG 2. Histologic analysis of AD in DS-Nh mice vaccinated with MIF/TTX or pCAGGS plasmid before disease onset. Nine-week-old DS-Nh mice without eruptions were administered MIF/TTX or control plasmid. Specimens were collected from the dorsal skin 6 weeks after the first vaccination and stained with hematoxylin 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.

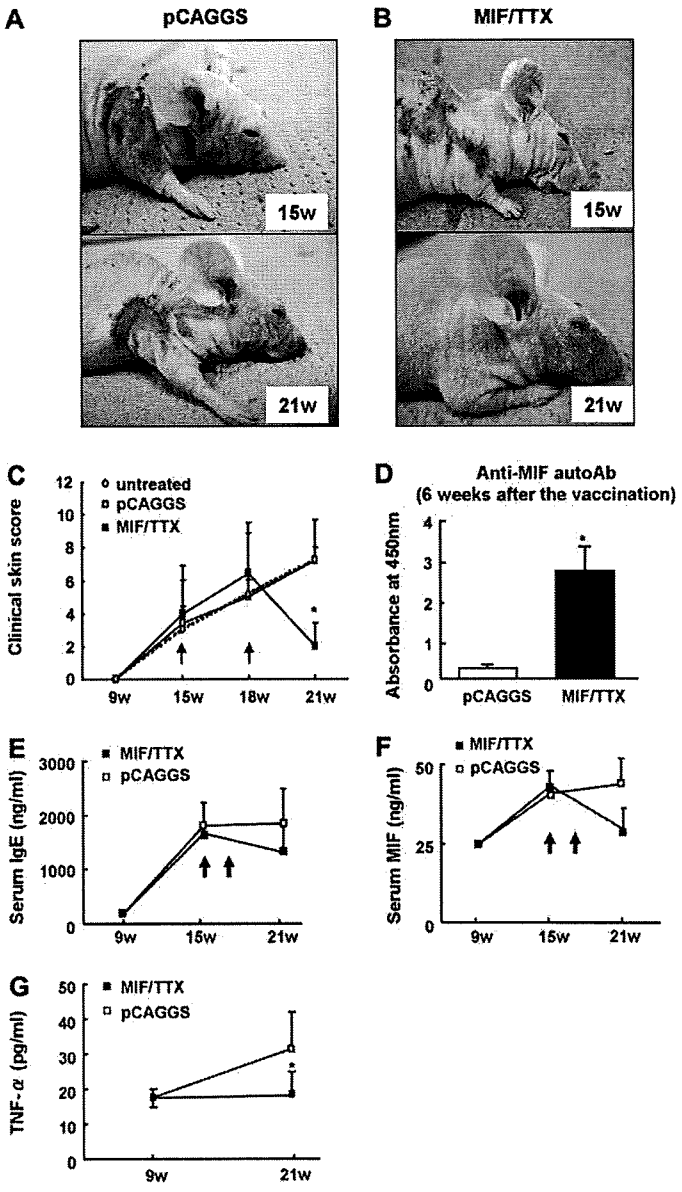

FIG 3. Therapeutic effect of MIF-DNA vaccination in DS-Nh mice with pre-existing AD. Fifteen-week-old DS-Nh mice with ongoing dermatitis were administered MIF/TTX or control plasmid (pCAGGS) or left untreated. **A**, Clinical features of DS-Nh mice vaccinated with control plasmid. **B**, Clinical features 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 anti-cytokine 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

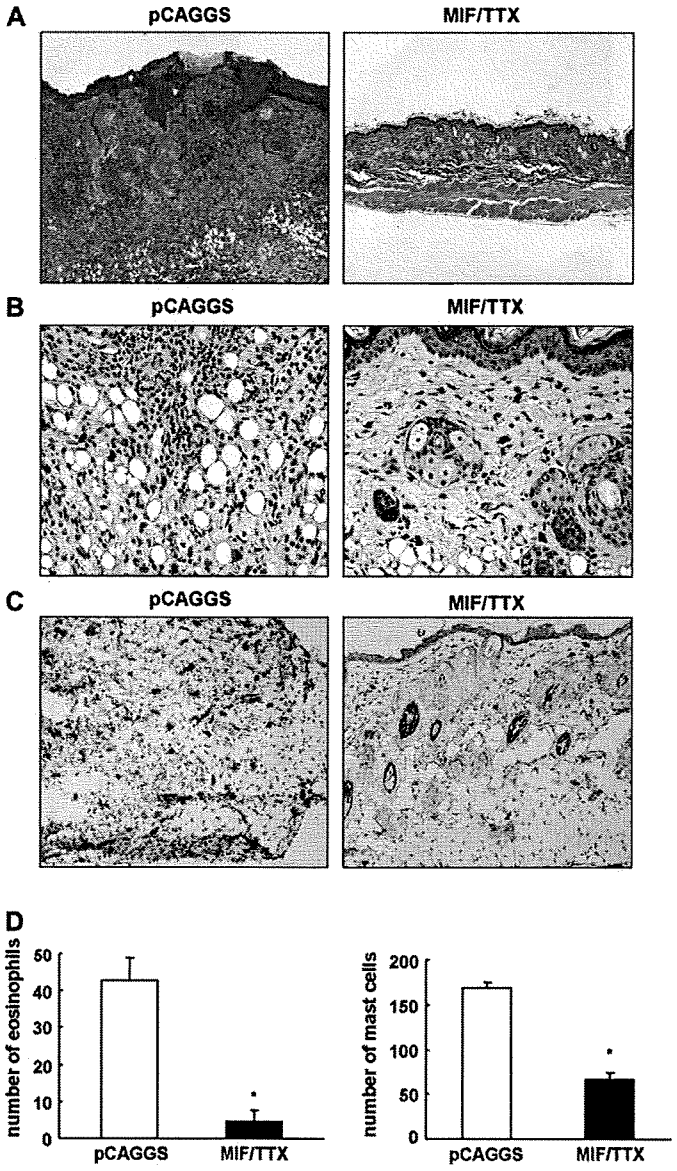

FIG 4. Histologic analysis of AD in DS-Nh mice vaccinated with MIF/TTX or control plasmid after disease onset. Fifteen-week-old DS-Nh mice with ongoing dermatitis were administered MIF/TTX or control (pCAGGS) plasmid. Specimens were collected at 6 weeks after the first vaccination and stained with hematoxylin 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

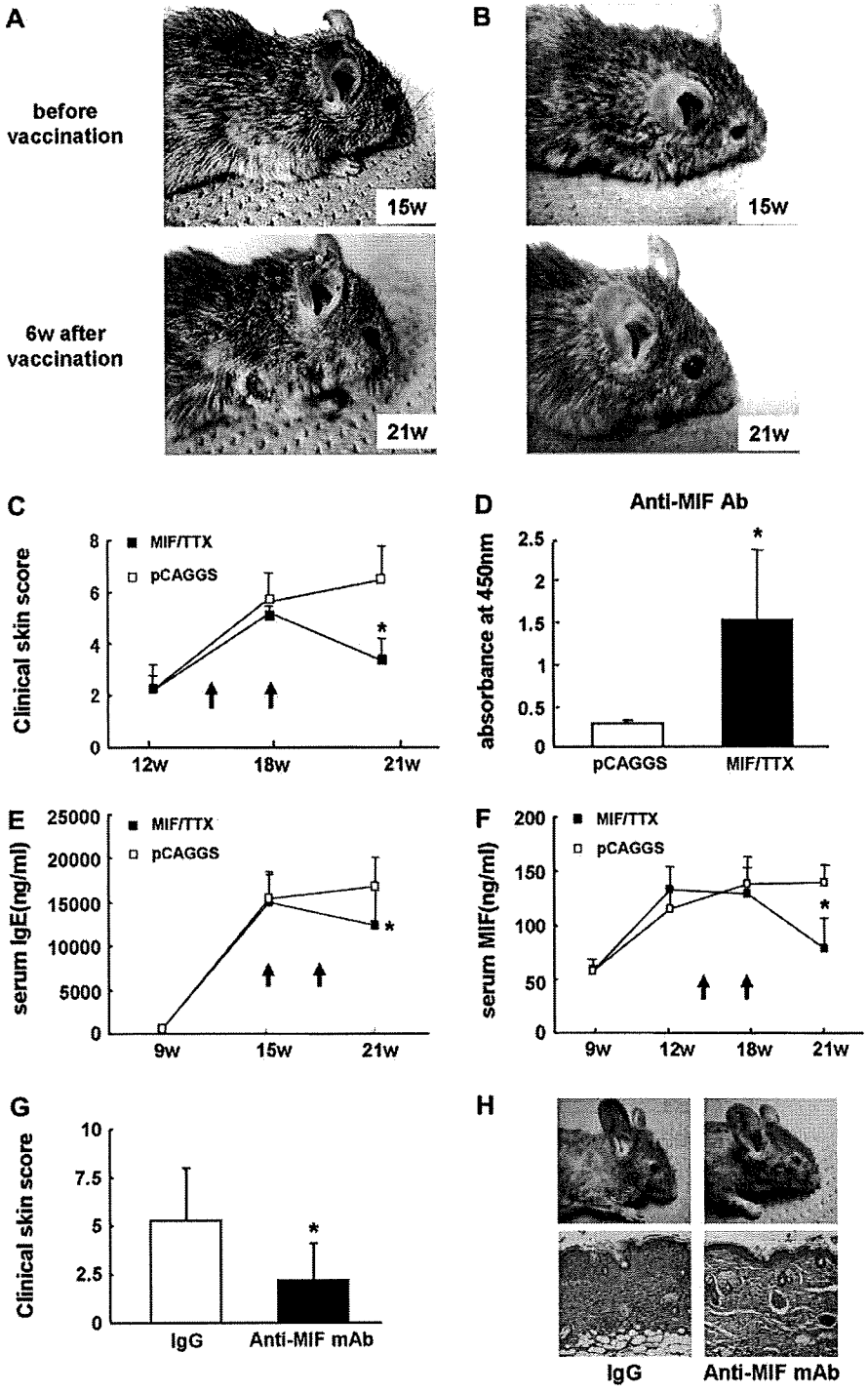

FIG 5. Therapeutic effect of MIF/TTX-DNA in vaccine for pre-existing AD on NC/Nga mice. Fifteen-week-old NC/Nga mice with dermatitis were vaccinated with control pCAGGS plasmid or MIF/TTX. **A**, Clinical features of NC/Nga mice vaccinated with control pCAGGS plasmid. **B**, Clinical features of DS-Nh 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.

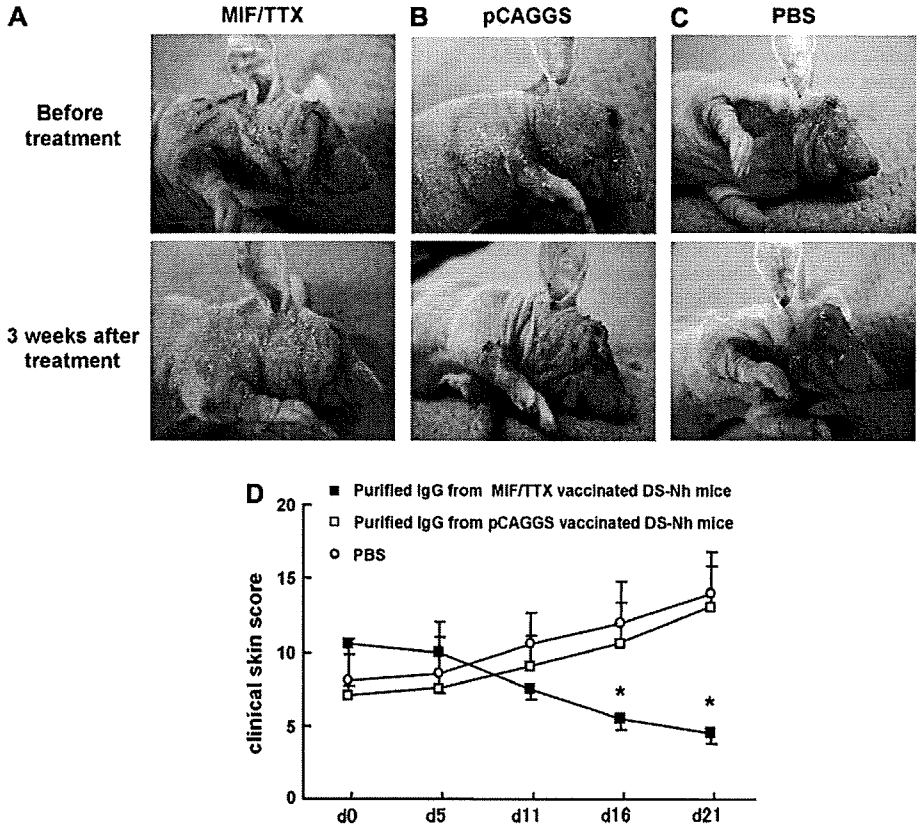

FIG 6. Adoptive transfer of autoantibodies elicited by DNA vaccines. IgG were purified from the sera of control (pCAGGS) plasmid– or MIF/TTX-vaccinated DS mice and adoptively transferred. DS-Nh mice were administered 50 μg per mouse of purified IgG from control plasmid–vaccinated DS-Nh mice, purified 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.

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

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Case Report

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

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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.

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

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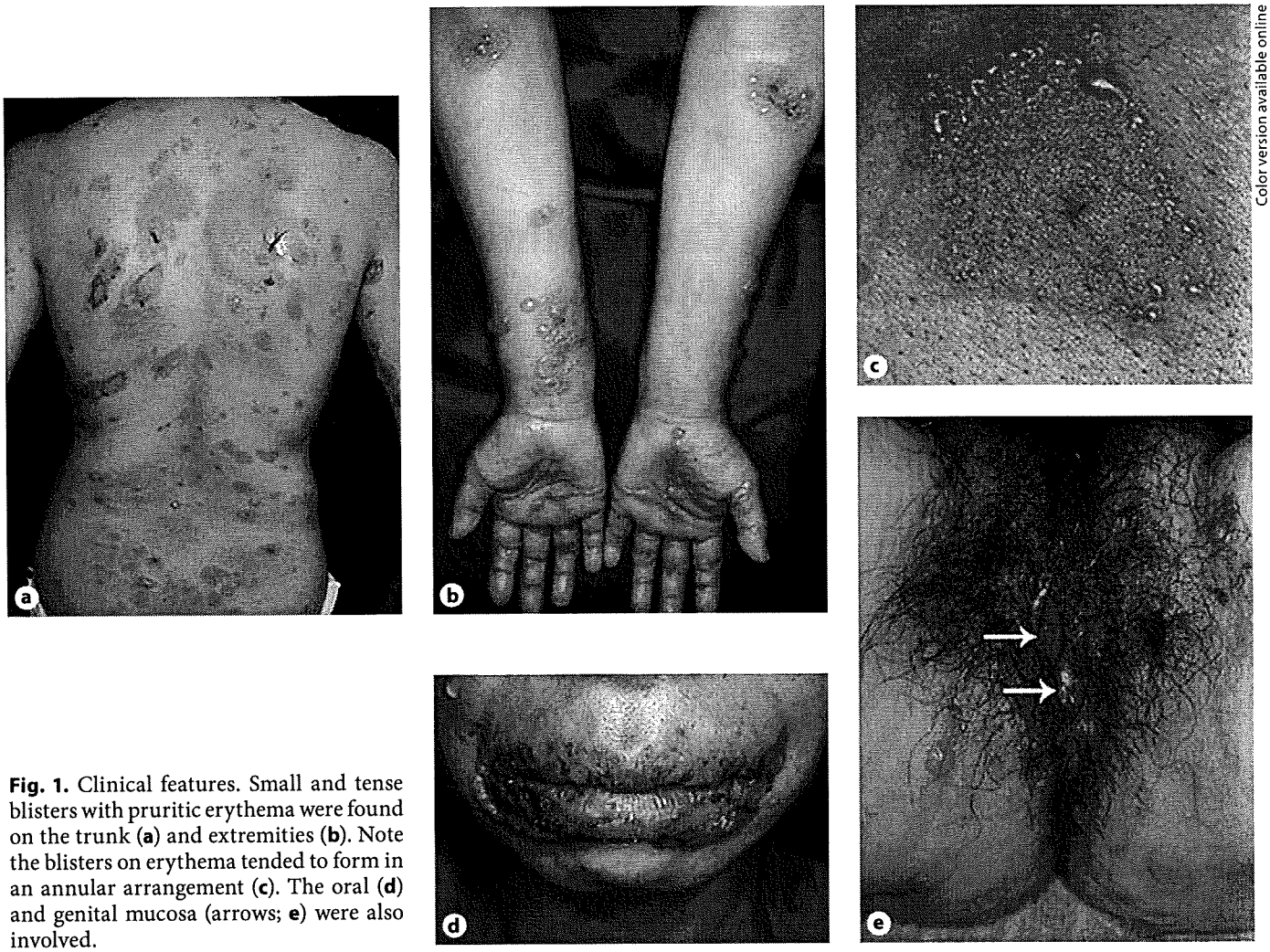

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Fig. 1. Clinical features. Small and tense blisters with pruritic erythema were found on the trunk (**a**) and extremities (**b**). Note the blisters on erythema tended to form in an annular arrangement (**c**). The oral (**d**) and genital mucosa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

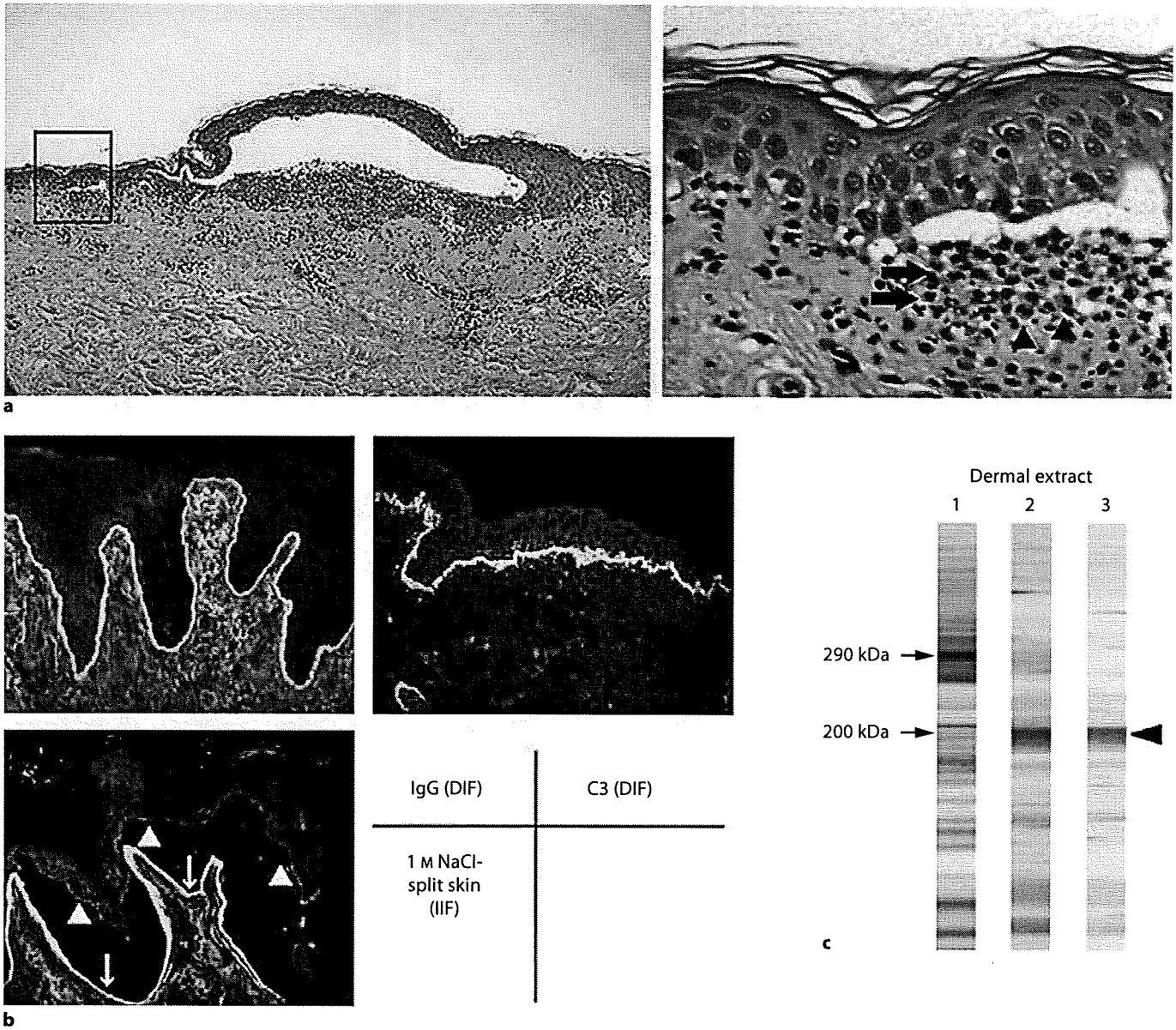

Fig. 2. Histopathology. **a** A subepidermal blister with numerous infiltrating neutrophils (arrows) and eosinophils (arrowheads) was observed. **b** A direct immunofluorescence study demonstrated linear in vivo deposition of IgG and C3 along the epidermal basement membrane. An indirect immunofluorescence 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

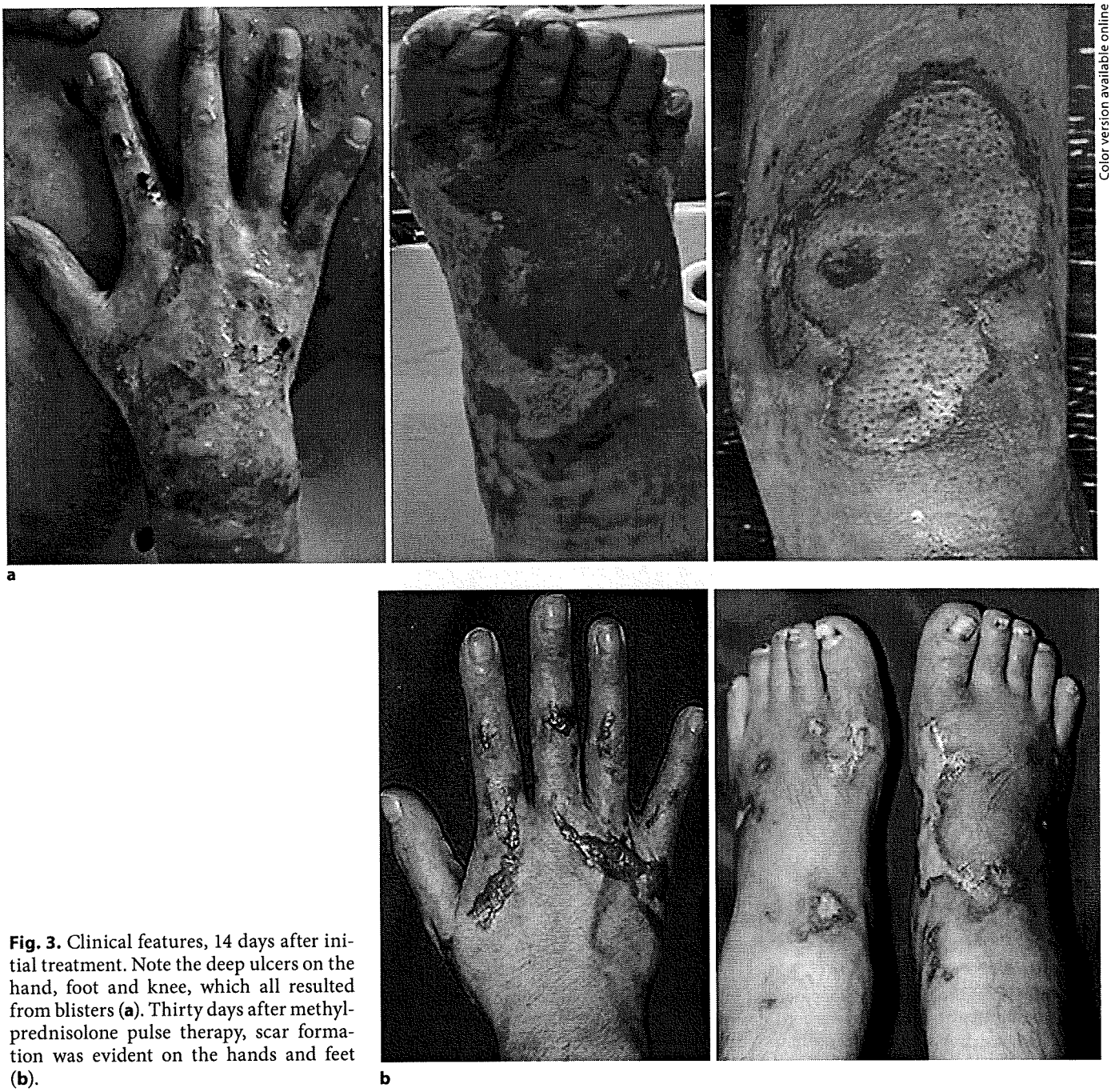

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Fig. 3. Clinical features, 14 days after initial treatment. Note the deep ulcers on the hand, foot and knee, which all resulted from blisters (**a**). Thirty days after methylprednisolone pulse therapy, scar formation was evident on the hands and feet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