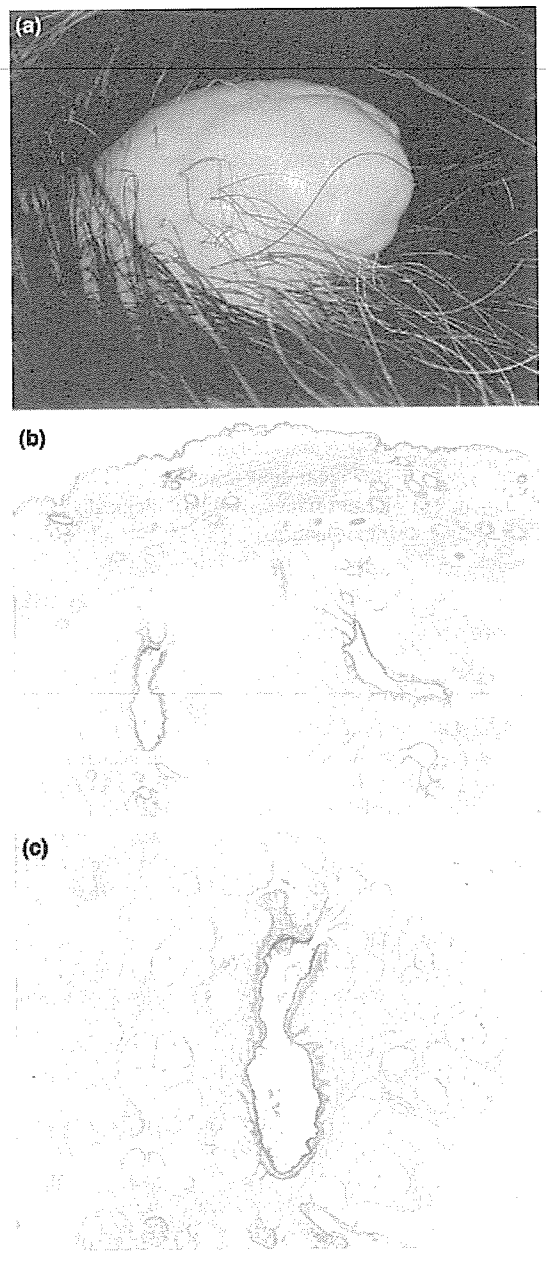

Figure 1 (a) A normal skin-coloured, 25-mm asymptomatic nodule with a smooth skin surface; (b) low-power view showing a dilated folliculosebaceous unit; (c) multiple sebaceous lobules radiate outward from the cystic structure, predominantly showing infundibular keratinization. Haematoxylin and eosin; original magnification (b) × 6; (c) × 40.

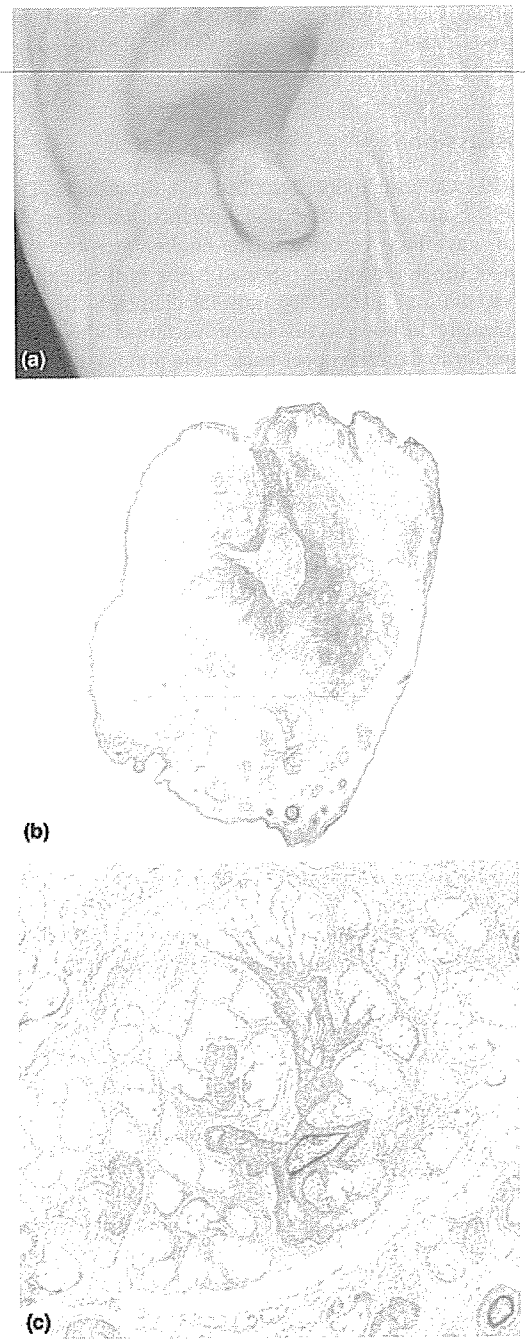

Figure 2 (a) Normal skin-coloured, 10-mm pedunculated nodule on the right auricle; (b) excess fibrous components (around the cystic structure and sebaceous lobules; (c) clefts can be seen between the pericystic fibrous tissue and the rest of the dermis. Haematoxylin and eosin, original magnification (b) ×4; (c) ×40).

glands. Clefts were observed between the pericystic fibrous tissue and the surrounding dermis. These features were characteristic of FSCH.

Discussion

FSCH was originally described by Kimura *et al.* in 1991,[1] and since then, 34 patients, including our two, have been reported in the English literature. The size of the lesions in all reported cases on the head and neck did not exceed 25 mm in diameter. In three extremely large growths (giant variants), the lesions were located on the upper back, labia majora and upper arm.[2–4]

Physical examination shows FSCH lesions to be asymptomatic, usually rubbery to firm in consistency. They tend to grow slowly with no change in colour or texture over time.[5] FSCH lacks distinctive clinical features, and the initial diagnoses in all reported cases included disorders other than FSCH, such as intradermal naevus, sebaceous hyperplasia, basal cell carcinoma, lipoma, and neurofibroma.[5] Our two cases were misdiagnosed as neurofibroma and soft fibroma, respectively.

Histopathological features of FSCH include: (i) an infundibular cystic structure adjacent to the sebaceous glands; (ii) laminated fibroplasia around the epithelial component; (iii) mesenchymal changes around these fibroepithelial units (collagen bundles, adipocytes and small venules); (iv) clefts between the fibroepithelial units and the surrounding altered stroma, and the adjacent normal skin structures; (v) confinement of these processes primarily to the dermis.[1]

FSCH is sometimes difficult to differentiate from trichofolliculoma, especially those variants with marked sebaceous components, termed sebaceous trichofolliculoma (STF) by Plewig.[6] Although both exhibit a widely dilated cavity or sinus lined by stratified squamous epithelium with numerous sebaceous lobules, there are many differential points. Clinically, STF is a centrally depressed lesion that exhibits protruding hairs of nasal apex and tends to occur at a young age, often in neonates. Histopathologically, STF contains numerous hair follicles, with the lower portion composed of terminal hairs, vellus hairs and trichoids arising from the cyst wall. In addition, no mesenchymal changes are found in STF. Recently, Schulz and Hartschuh have proposed that FSCH could actually be a late stage in trichofolliculoma formation.[7] However, their conclusion fails to explain congenital FSCH cases sufficiently;[2,5,8] the idea of FSCH being a late stage of STF with mesenchymal changes is difficult to reconcile with its documented appearance in neonates.

Interestingly, several cases of FSCH with abnormal neural components have been reported.[5,9] These variations indicate that the FSCH is intrinsically a skin hamartoma including mesenchymal differentiation, which is not seen in STF.

FSCH usually occurs on the head or neck as a single, nodular lesion usually < 25 mm in diameter. Clinical diagnosis of FSCH is remarkably difficult because it can have a varying clinical appearance, in many cases similar to intradermal naevi. Histopathologically, FSCH shares several similar features with STF, but it is usually possible to differentiate these two tumours.

References

1 Kimura T, Miyazawa H, Aoyagi T, Ackerman AB. Folliculosebaceous cystic hamartoma. A distinctive malformation of the skin. *Am J Dermatopathol* 1991; **13**: 213–20.
2 Yamamoto O, Suenaga Y, Bhawan J. Giant folliculosebaceous cystic hamartoma. *J Cutan Pathol* 1994; **21**: 170–2.
3 Bolognia JL, Longley BJ. Giant folliculosebaceous cystic hamartoma. *Dermatology* 1998; **197**: 258–60.
4 Sturtz DE, Smith DJ, Calderon MS, Fullen DR. Giant folliculosebaceous cystic hamartoma of the upper extremity. *J Cutan Pathol* 2004; **31**: 287–90.
5 Mohammad A, Salonas A, Mona R *et al.* Folliculo-sebaceous cystic hamartoma. *Int J Dermatol* 2001; **40**: 454–7.
6 Plewig G. Sebaceous trichofolliculoma. *J Cutan Pathol* 1980; **7**: 349–403.
7 Schulz T, Hartschuh W. Folliculosebaceous cystic hamartoma is a trichofolliculoma at its very late stage. *J Cutan Pathol* 1998; **25**: 354–64.
8 Aloi F, Tomasini C, Pippione M. Folliculosebaceous cystic hamartoma with perifollicular mucinosis. *Am J Dermatopathol* 1996; **18**: 58–62.
9 Toyoda M, Morohashi M. Folliculosebaceous cystic hamartoma with a neural component: an immunohistochemical study. *J Dermatol* 1997; **24**: 451–7.

Morphology

Coexistence of a systemic lupus erythematosus and porphyria cutanea tarda: case successfully improved by avoidance of sun exposure

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Case Report

A 46-year-old Japanese woman presented with vesicles on her nose and the dosal aspect of her hands since 3 months. Physical examination revealed tense blisters and atrophic erythematous plaques on her arms and the dosal side of her hands (Fig. 1a). Erosions were also scattered on the face (Fig. 1b). She also had hypertrichosis on her face. The patient was first diagnosed as having systemic lupus erythematosus (SLE) in 1997 at the age of 41 years, when she presented with alopecia, fever, arthralgia, Raynaud's phenomenon, hemolytic anemia and lymphadenopathy. Liver transaminase levels were slightly elevated. She had no sign of hepatitis including autoimmune hepatitis, drug-related hepatotoxity, alcoholic hepatitis, or viral infection. Systemic lupus erythematosus was well-controlled with a daily 5-mg dose of prednisolone (PSL).

At age of 43 years she developed erythematous plaques with scaling on the back of her hands. Clinically the skin eruptions at that time appeared to be discoid lupus erythematodes. She was treated with a steroid ointment and her eruptions gradually improved, but new skin lesions repeatedly appeared on her hands.

Laboratory investigations at age 46 years while taking PSL 5 mg disclosed the following results: white cell count 3.9×10^9/l with lymphopenia of 0.78×10^9/l, red cell count 3.35×10^{12}/l, hemoglobin of 11.4 g/l, and a platelet count of 165×10^9/l. Liver function profile included total bilirubin was 1.7 mg/dl, GOT 46 IU/l, GPT 82 IU/l, γ-GTP 142 IU/l, and alkaline phosphatase 456 IU/l. Renal function and electrolytes were normal. Antinuclear antibody (ANA) titers were positive at a dilution of 1/80 (normal value, 1/40) with a homogeneous pattern. Histopathological examination of the back of the hands demonstrated a subepidermal blister with slight inflammatory infiltrate. No eosinophilic deposit around the vessels and little edematous change was present in the dermis (Fig. 2). Direct immunofluorescence revealed a granular fashion at the dermoepidermal junction (DEJ) with IgG, IgM, and C_3. Deposition of C_3 around the blood vessels and several clusters of colloid bodies associated with IgM were also observed in the upper dermis. Laboratory value of fractionated porphyrins in her urine revealed the following: uroporphyrin 1080 μg/l (normal: < 20), coproporophrin 110 μg/L (normal < 100), porphobironogen 1.3 mg/l (normal < 2), and δ-aminolevulinic acid 3.5 mg/dl. The value of porphyrins in her serum was within the normal range, confirming the diagnosis of PCT. Subsequently, she was treated with topical steroid ointment, and avoidance of sun exposure was implemented. Three months later the value of uroporphyrin and coproporphirin in her urine was almost normal and no further blisters appeared.

Discussion

Since the association of SLE and PCT was first described by Linden in 1954,[1] approximately 50 cases of LE (all variants) have been described in association with PCT, including 15 cases reported by Gibson and McEvoy.[2] PCT results from

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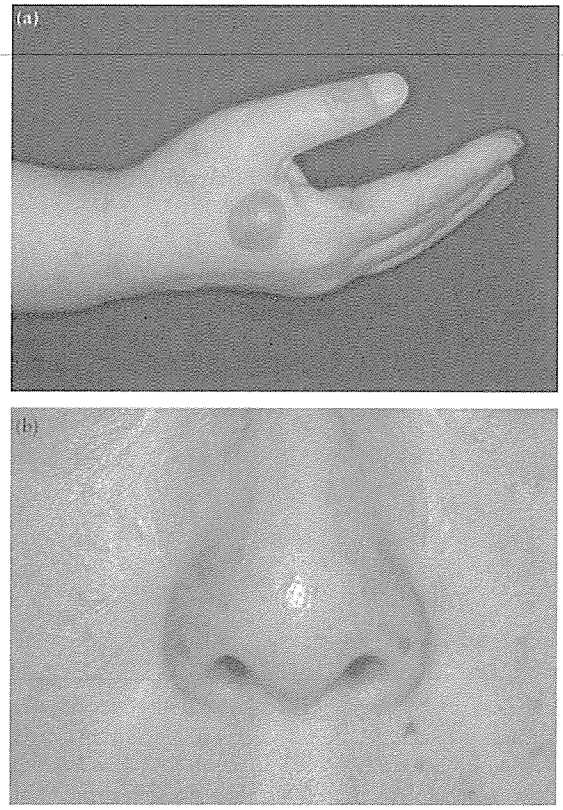

Figure 1 (a) Right hand with a tense blister, erosions and several pigmented lesions. (b) Numerous erosions on the face

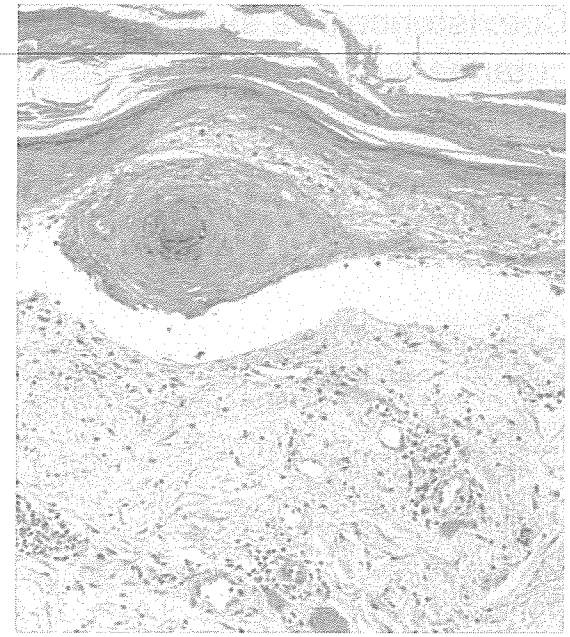

Figure 2 Subepidermal blister with slight inflammatory infiltrate. No eosinophilic deposits are observed around the vessels and few edematous changes were present in the dermis (H&E ×40)

decreased activity of uroporphyrinogen decarboxylase (UROD). Alcohol, oral contraceptives, polychlorinated hydrocarbons, disturbances of iron metabolism, hepatitis C and infection with HIV are recognized as precipitant factors for PCT.[3] Approximately 80% of PCT patients have the sporadic form in which UROD deficiency is restricted to the liver and the remainder has the familial type in which mutations in the UROD gene are inherited in an autosomal dominant pattern.[4]

It is not known whether the association between PCT and LE is coincidental or represents some common link. Harris *et al.* have suggested possible explanations for the coexistence of LE and porphyria: a common genetic abnormality, porphyria triggering an autoimmune response, preexisting LE resulting in an acquired metabolic fault leading to porphyria, and LE precipitating a genetically determined metabolic fault resulting in porphyria.[5] It is interesting that the gene for UROD is located on chromosome 1,[6] and that the 1q41–q42 region of that chromosome is probably linked to SLE.[7]

Our patient did not drink much alcohol, take oral contraceptives, nor had any blood transfusions. She did not have HCV or HIV infections. Her liver function test was abnormal, and therefore dysfunction of her liver caused by SLE may have been a factor in precipitating the PCT. She had suffered from hemolytic anemia since 1997, so an overload of iron may have caused liver dysfunction and could also have precipitated the PCT. The histological findings of the subepidermal blistering are compatible with PCT, and positive immunofluorescence staining at the DEJ may have been caused by the SLE and sun-exposed skin.

Association of SLE and PCT is not common, and this combination usually poses practical problems and occasionally therapeutic dilemmas. Phlebotomy is one treatment for PCT, but it is not advisable in LE patients who have anemia. Antimalarials are used for the treatment of LE and PCT. Severe toxicity can result from using high doses of chloroquine,[8] and hydroxichloroquine may also be associated with abdominal crises in some cases.[9] However, low-dose chloroquine therapy has been shown to be effective without causing serious hepatotoxicity.[10] Therefore, it is important to avoid high-dose chloroquine therapy in cases of joint LE and PCT.

Our patient's skin lesion and the quantity of porphyrines in her urine have been improved since she started the avoidance of sun exposure together with the topical steroid ointment treatment. Strict total sun protection should be recommended

to patients with PCT and SLE because both conditions can be activated by long wavelength light bound in sunlight.

References

1 Linden IH, Steffen CG, Newcomer VD, *et al.* Development of porphyria during chloroquine therapy for choronic discoid lupus erythmatosus. *Calif Med* 1954; 81: 235–238.

2 Gibson GE, McEvoy MT. Coexistence of lupus erythematosus and porphyria cutanea tarda in fifteen patients. *J Am Acad Dermatol* 1998; 4: 569–573.

3 O'Connor WJ, Murphy GM, Darby C, *et al.* Porphyrin abnormalities in acquired immunodeficiency syndrome. *Arch Dermatol* 1996; 132: 1443–1447.

4 Elder GH. Porphyriacutanea tarda. *Semin Liver Dis* 1998; 18: 67–75.

5 Harrris MY, Mills GC, Levin WC. Coexistent systemic lupus erythematosus and porphria. *Arch Intern Med* 1966; 117: 425–428.

6 Wu C, Xu W, Kozack CA, *et al.* Mouse uroporphyrinogen decarboxylase. cDNA cloning, expression, and mapping. *Mamm Genome* 1996; 7: 349–352.

7 Tsao BP, Cantor RM, Kalunion KC, *et al.* Evidence for linkage of a candidate chromosome I region for human systemic lupus erythematosus. *J Clin Invest* 1997; 99: 725–731.

8 Felsher BF, Redeker AG. Effect of chloroquine on hepatic uroporphrin metabolism in patients with porphyria cutanea tarda. *Medicine (Balt)* 1966; 45: 575–583.

9 Koranda FC. Antimalarials. *J Am Acad Dermatol* 1981; 4: 650–655.

10 Taljaard JJ, Shanley BC, Stewart-Wynne EG, *et al.* Studies on low dose choloroquine therapy and the action of chloroquine in symptomatic porphria. *Br J Dermatol* 1972; 87: 261–269.

Positive Comments Made by Patients:

- "I get the feeling that I have a more active role and I like that. I am more aware of my complaint and my expectations."
- "I don't know who this person (teledermatologist) is, but I assume he has enough knowledge and experience to provide a good judgment."
- "You have to describe your complaint in detail, which can probably improve the diagnostics."
- "This service is accessible 24 hours, 7 days a week."
- "It is distant (pleasant)."

Negative Comments Made by Patients:

- "I don't speak Latin."
- "I really think somebody should see it face-to-face."
- "It's only a judgment from the images."
- "The answers are short and impersonal."

Figure. Positive and negative comments made by patients as an explanation for their scores.

after the teleconsultation, which was higher than before the teleconsultation (71%; 60 of 84). Patients were as positive about a teledermatologist providing a diagnosis, treatment plan, and detailed information before the teleconsultation as after.

Half of the patients (42 of 84) were willing to pay up to €25 for the service; 24% (20 of 84) were willing to pay between €26 and €50 for a consultation; 20% (17 of 84) were willing to pay between €51 and €100; and 6% (5 of 84) would have paid more than €100.

Comment. In general, patients responded positively to patient-assisted teledermatology. A quick response from the teledermatologist was considered an important advantage. A disadvantage was the remote character of the service with lack of personal interaction. However, this remoteness might be seen as an advantage by some patients, for example those who are shy or embarrassed.

To our knowledge, this is the first study on this type of patient-assisted teleconsultation service with a secondary care provider. Internet communication in health care is becoming more accepted and more frequently initiated by patients.[4,5] It is important to know patient perceptions and willingness to pay before such services are introduced.

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1. Eedy DJ, Wootton R. Teledermatology: a review. *Br J Dermatol.* 2001;144:696-707.
2. Burdick AE, Berman B. Teledermatology. *Adv Dermatol.* 1997;12:19-45.
3. Eminovic N, Witkamp L, Ravelli AC, et al. Potential effect of patient-assisted teledermatology on outpatient referral rates. *J Telemed Telecare.* 2003;9:321-327.
4. Car J, Sheikh A. Email consultations in health care: 2-acceptability and safe application. *BMJ.* 2004;329:439-442.
5. Borowitz SM, Wyatt JC. The origin, content, and workload of e-mail consultations. *JAMA.* 1998;280:1321-1324.

VIGNETTES

Peginterferon Alfa-2b for Mycosis Fungoides

Currently, peginterferon alfa-2b plus ribavirin has been shown to be the best available therapy for patients with chronic hepatitis C virus (HCV) infection.[1] Peginterferon alfa-2b (a 12-kDa linear polyethylene glycol moiety) plus ribavirin produces a significantly improved and sustained antiviral response compared with interferon alfa-2b plus ribavirin.[1] Peginterferon alfa-2b has an extended serum half-life that provides constant viral suppression for 7 days, thus allowing once-weekly dosing and an enhanced clinical efficacy.[1] While interferon alfa-2b has been used to treat T-cell lymphoma,[2,3] we report herein the significant efficacy of peginterferon alfa-2b for treatment of skin lesions caused by mycosis fungoides (MF).

A 67-year-old Japanese man was referred to our hospital with a 6-month history of multiple, asymptomatic brown-red–colored scaly erythematous macules and plaques over his arms and legs (**Figure**, A and B). He had not received any medications prior to the onset of his skin eruption. A skin biopsy specimen from his lower leg showed epidermotropism and dense upper dermal infiltration of atypical lymphocytes, which is consistent with MF (Figure, C). T-cell receptor rearrangement (both Jγ and Cβ chains) were recognized in his biopsy specimens. Peripheral blood analysis revealed a normal flow cytometric pattern. Biochemical tests revealed high soluble interleukin (IL) 2 receptor (1795 U/mL; normal range, 135-483 U/mL); however, his human T-cell lymphotropic virus type 1 antibody was negative. After complete systemic examination, including enhanced computed tomography, gallium scintigraphy, and bone marrow aspiration test, no internal invasion was detected. We confirmed the diagnosis of MF plaque stage. We started treatment with topical steroid ointment, systemic psoralen UV-A (PUVA), and etretinate. However, the lesions had been refractory against this treatment for approximately 5 months.

Our patient also had chronic HCV infection, and laboratory data showed an aspartate aminotransferase level of 33 IU/L, an alanine aminotransferase level of 19 IU/L, a total bilirubin level of 0.9 mg/dL (15.4 μmol/L), and an HCV RNA level greater than 5000 kIU/mL. He started treatment for chronic HCV infection with peginterferon alfa-2b plus ribavirin. Peginterferon alfa-2b (80 μg) was injected subcutaneously once a week, and oral ribavirin, 600 mg, was administered daily. At week 28, his serum HCV RNA level had decreased, so his therapy was continued.

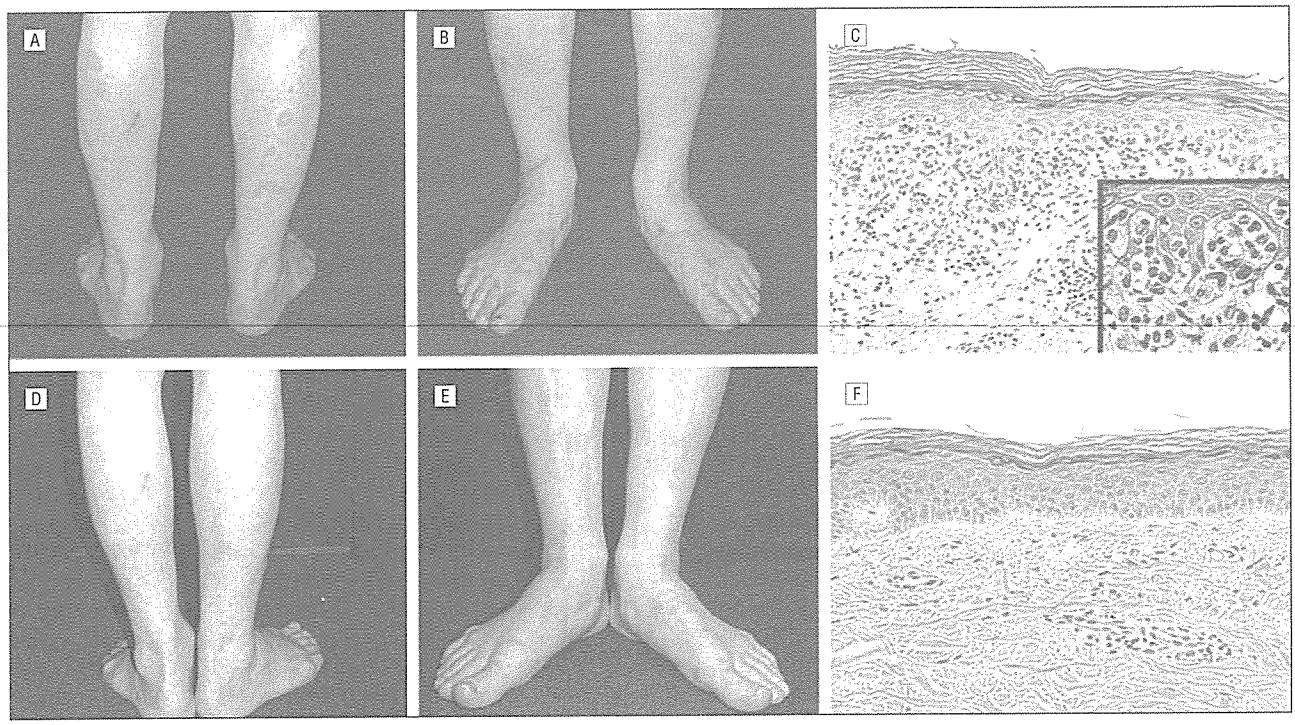

Figure. Mycosis fungoides skin lesions before the treatment with peginterferon alfa-2b (A and B) and after 4 weeks of therapy (D and E). Light microscopic finding of a mycosis fungoides skin lesion before treatment with peginterferon alfa-2b (C) (inset [original magnification ×100] shows the atypical lymphocytes) and after 4 weeks of therapy (F) (hematoxylin-eosin, original magnification ×40).

Interestingly, soon after the initiation of peginterferon alfa-2b, not only the HCV infection but also the patient's skin lesions showed a dramatic improvement, and in only 4 weeks most of the skin lesions had clinically subsided (Figure, D and E). When peginterferon alfa-2b and ribavirin treatments were started, he had been receiving treatment with oral etretinate (10 mg/d) and systemic PUVA (1.3 J once weekly) over a 3-month period, but we stopped these treatments at week 4 because of his skin improvement. To further evaluate whether his MF skin lesions had improved at the microscopic level, we performed a skin biopsy at the same site as the previous biopsy. While dense atypical lymphocyte infiltrates were present in the previous biopsy specimen, no significant numbers of atypical lymphocytes were recognized in the later biopsy specimen (Figure, F). Biochemical tests revealed that the level of soluble IL-2 receptor was decreased (1107 U/mL at week 20). Recurrence of MF lesions was not noted during the treatment period. He has remained in remission and off all skin therapy for the past 24 weeks.

The efficacy of interferon alfa-2b has been well reported and includes MF treatment.[2,3] Interferon alfa has been shown to inhibit IL-4 and IL-5 production by T cells in patients with Sézary syndrome.[3,4] Interferon molecules have been shown to induce cell-mediated cytotoxic effects and stimulate natural killer cell activity in vivo.[3] In addition, interferon alfa-2a plus PUVA or extracorporeal phototherapy have been reported.[5,6] On the other hand, peginterferon alfa-2b and ribavirin treatment of HCV-related, low-grade, B-cell, non-Hodgkin lymphoma has been reported.[7] Although it is reported that MF is not associated with HCV infection today, our patient's improvement might be related with his HCV infection.[8] Moreover, peginterferon is known to exert immunomodulatory and antiangiogenic effects and has been used to treat multiple myeloma.[9]

Peginterferon alfa-2b is a new drug for HCV treatment, and therefore its effects on MF have not been well studied. To our knowledge, this is the first MF report in which the marked efficacy of peginterferon alfa-2b has been confirmed both clinically and histopathologically. This example of 1 case cannot provide evidence whether peginterferon is superior to standard interferon treatment, but it does suggest that further studies are warranted.

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1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet*. 2001;358:958-965.
2. Knobler RM, Trautinger F, Radaszkiewicz T, et al. Treatment of cutaneous T cell lymphoma with a combination of low-dose interferon alfa-2b and retinoids. *J Am Acad Dermatol*. 1991;24:247-252.

3. McGinnis KS, Junkins-Hopkins JM, Crawford G, et al. Low-dose oral bexarotene in combination with low-dose interferon alfa in the treatment of cutaneous T-cell lymphoma: clinical synergism and possible immunologic mechanism. *J Am Acad Dermatol.* 2004;50:375-379.
4. Suchin KR, Cassin M, Gottleib SL, et al. Increased interleukin 5 production in eosinophilic Sezary syndrome: regulation by interferon alfa and interleukin 12. *J Am Acad Dermatol.* 2001;44:28-32.
5. Chiarion-Sileni V, Bononi A, Fornasa CV, et al. Phase II trial of interferon-alpha-2a plus psolaren with ultraviolet light A in patients with cutaneous T-cell lymphoma. *Cancer.* 2002;95:569-575.
6. Wollina U, Looks A, Meyer J, et al. Treatment of stage II cutaneous T-cell lymphoma with interferon alfa-2a and extracorporeal photochemotherapy: a prospective controlled trial. *J Am Acad Dermatol.* 2001;44:253-260.
7. Vallisa D, Bernuzzi P, Arcaini L, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. *J Clin Oncol.* 2005;23:468-473.
8. Miertusova S, Bonin S, Trevisan G, Stanta G. Mycosis fungoides is not associated with hepatitis C virus infection. *Br J Dermatol.* 2004;151:1108-1110.
9. Kasper B, Moehler T, Neben K, Ho AD, Goldschmidt H. Combination therapy of thalidomide and peginterferon in patients with progressive multiple myeloma. *Ann Oncol.* 2004;15:176-177.

Oral Involvement in Hydroa Vacciniforme

Hydroa vacciniforme (HV) is a rare photosensitivity disorder with onset in childhood. Oral mucosal involvement seems to be rare. We report a case of a 14-year-old boy who had a recurrent papulovesicular necrotic facial eruption. One year prior to presentation at our clinic, his skin symptoms appeared after sun exposure. The eruption resolved in a few weeks with some varicellalike scars. After he used strict photoprotection (Photoderm Max; Bioderma, Lyon, France), there was no recurrence of his cutaneous disorder. In May 2005, he was referred to our clinic with a new relapse of his skin rash triggered by 2 days of sun exposure without any photoprotection. He complained of severe mouth and lip pain. There were no symptoms of ocular involvement.

At clinical examination, he had multiple clusters of confluent necrotic papulovesicles located on his nose and cheeks (**Figure**, A). He also had necrotic papulovesicles and flesh-colored papules on his ears and neck. His lower lip was edematous and covered with large confluent crusted ulcers extending to the lower vestibule. There were no papillae on the distal atrophic mucosa on his tongue (Figure, B). Findings from his general examination were otherwise normal. Vaccinialike lesions with a necrotic and vesiculopustular aspect as well as relation with sun exposure were highly suggestive of HV.

Histological examination of one of the neck papules showed an inflammatory perivascular infiltrate made of lymphocytes. There was no evident sign of lymphoproliferative disorder. Antinuclear factors were negative. Epstein-Barr virus serologic examination revealed a former infection. The patient was treated with hydroxychloroquine sulfate, 800 mg daily, and he was encouraged to apply a photoprotective cream. The skin and mucosal involvement resolved within 2 weeks.

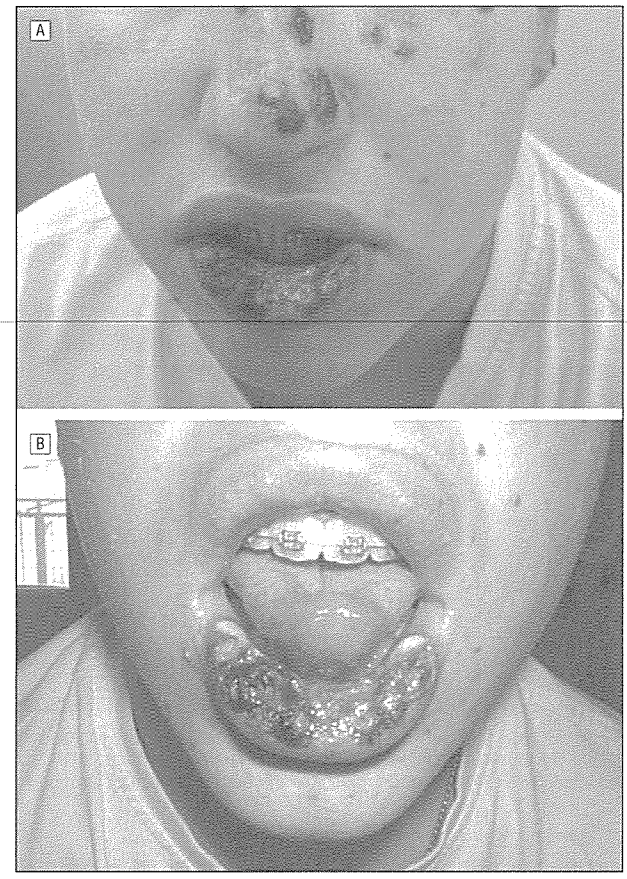

Figure. A, Necrotic papulovesicles located on the nose and cheeks; B, crusted lip ulcers extending to the lower vestibule.

Mucous involvement in HV, which is rare, mostly occurs only on the photoexposed lower lip. In this case, the mucous involvement appears to be a result of contiguous extension of sun-exposed lesions rather than being a distinct site of HV. To our knowledge, only 1 case of mouth ulcers in a 6-year-old girl has been previously described.[1] While the physiopathologic mechanism of HV is not clear, it has been suggested recently that HV could be due to the clonal natural killer cells with latent Epstein-Barr virus infection.[2]

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Financial Disclosure: None.

1. Yesudian PD, Sharp GR. Hydroa vacciniforme with oral mucosal involvement. *Pediatr Dermatol.* 2004;21:555-557.
2. Demachi A, Nagata H, Morio T, et al. Characterization of Ebstein-Barr virus (EBV)-positive NK cells isolated from hydroa vacciniforme-like eruptions. *Microbiol Immunol.* 2003;47:543-552.

eosinophils (816/μL). Blood chemistry values were normal except for lactate dehydrogenase level (331 IU/mL; normal range, 119-229 IU/mL). A skin biopsy specimen showed moderate perivascular infiltration of lymphocytes in the upper dermis with exocytosis into the epidermis (Figure). Most of the lymphocytes were CD4 positive. T-cell monoclonality, as assessed by Southern blot analysis of T-cell receptor Cβ1, was absent.

Aspirin therapy was discontinued, and he was treated with topical application of 0.1% mometasone furoate once a day for 2 months and narrowband UV-B irradiation (total dose, 19 600 mJ/cm^2) with clinical improvement. Although patch test results to the 2 drugs were negative, the lymphocyte stimulation test result was positive with aspirin, with a stimulation index of 4.48 (normal, <1.8). An oral challenge test result was positive with aspirin (20 mg, one fifth of the daily dose), as the patient developed the same erythematous papular eruption 24 hours after administration.

A flow cytometric analysis of the patient's peripheral blood lymphocytes was performed. The percentages of T_H1 and T_H2 cells were determined by the expression of chemokine receptors, CXCR3 and CCR4, respectively.[3] Twenty-four hours after the oral provocation test, the patient had a higher percentage of $CCR4^+$ $CD4^+$ T_H2 cells (36%) than $CXCR3^+$ $CD4^+$ T_H1 cells (8%). The percentage of $CD4^+$ cutaneous lymphocyte-associated antigen $(CLA)^+$ T cells was 12% at this time. After clinical improvement of the eruption, the percentages of $CCR4^+$ $CD4^+$ T_H2 cells and $CD4^+$ CLA^+ T cells were decreased to 28% and 1%, respectively. No recurrence was observed 14 months after cessation of aspirin.

Comment. Although the pathogenesis of papuloerythroderma remains unclear, a reaction to an unidentified cutaneous antigen has been suggested as well as the association with neoplasia.[2] Our case clearly demonstrated that aspirin can cause this eruption. The similar histologic findings to this case has been reported to be caused by other drugs under the title of pseudomycosis fungoides.[4,5] The known relationship of papuloerythroderma with mycosis fungoides further suggests that drugs are causative agents for papuloerythroderma.

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1. Ofuji S, Furukawa F, Miyachi Y, Ohno S. Papuloerythroderma. *Dermatologica.* 1984;169:125-130.
2. Wakeel RA, Keefe M, Chapman RS. Papuloerythroderma: another case of a new disease. *Arch Dermatol.* 1991;127:96-98.
3. Shimauchi T, Imai S, Hino R, Tokura Y. Production of thymus and activation-regulated chemokine and macrophage-derived chemokine by CCR4+ adult T-cell leukemia cells. *Clin Cancer Res.* 2005;11:2427-2435.
4. Gordon KB, Guitart J, Kuzel T, et al. Pseudo-mycosis fungoides in a patient taking clonazepam and fluoxetine. *J Am Acad Dermatol.* 1996;34:304-306.
5. Vermeer MH, Willemze R. Is mycosis fungoides exacerbated by fluoxetine? *J Am Acad Dermatol.* 1996;35:635-636.

Mycosis Fungoides Bullosa

Mycosis fungoides (MF), a cutaneous lymphoma with diverse clinical manifestations, rarely presents with vesiculobullous lesions, or MF bullosa (MFB). Mycosis fungoides with large cell transformation, transformed MF (T-MF), is also another rare condition. We report 2 cases of MFB, one of which was complicated with T-MF.

Report of Cases. *Case 1.* A 72-year-old woman, who was first diagnosed as having MF in her fifties (**Figure 1**A and B), presented with multiple nodules on her back (Figure 1C). Skin specimens of a nodule showed atypical cells with enlarged, pale nuclei clustered in the dermis (Figure 1D).These malignant cells stained positive for CD30. She was diagnosed as having T-MF and was treated with psoralen plus UV-A (PUVA) and topical corticosteroids.

At age 82 years, she presented with asymptomatic blisters measuring up to 10 mm in diameter, which were scattered over her right thigh (Figure 1E). She denied any history of insect bites, drug use, burns, or contact allergy. Within 5 months, the blisters resolved. A skin biopsy specimen from a blister revealed that the epidermis was separated from the dermis, and a bandlike infiltration of cells was seen (Figure 1F). The infiltrate was mainly composed of small atypical lymphocytes, intermingled with few histiocytes and eosinophils. Findings from immunohistochemical analysis showed that the infiltrate was predominantly composed of T cells (CD3 positive and CD30 negative). No large cells corresponding to T-MF were observed. Immunofluorescence revealed the absence of circulating autoantibodies against epidermis and no deposition of immunoglobulins and complements in vivo. We diagnosed the blistering lesions as MFB. The lesions disappeared after topical corticosteroid treatment and have not reappeared in the subsequent 2 years. She is still alive with lymph node and visceral involvement of MF.

Case 2. A 50-year-old woman with a 40-year history of early-stage MF visited our hospital after progression to tumor stage with multiple tumors over her whole body (**Figure 2**A). She was treated with topical corticosteroids, total electron beam irradiation, and 3 cycles of cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (CHOP) chemotherapy. Two months before her death, several vesicles appeared on her trunk (Figure 2B). She denied a history of insect bites, drug use, burns, or contact allergy. Histopathological findings from the vesicle showed subepidermal bulla with bandlike infiltration of atypical lymphocytes in the upper dermis (Figure 2C). She died of infection secondary to ulceration of tumor sites.

Comment. Bowman et al[1] proposed the following criteria for MFB diagnosis: (1) clinically apparent vesiculobullous lesions, (2) typical histologic features of MF with blisters, (3) negative immunofluorescence findings, and (4) exclusion of other causes of vesiculobul-

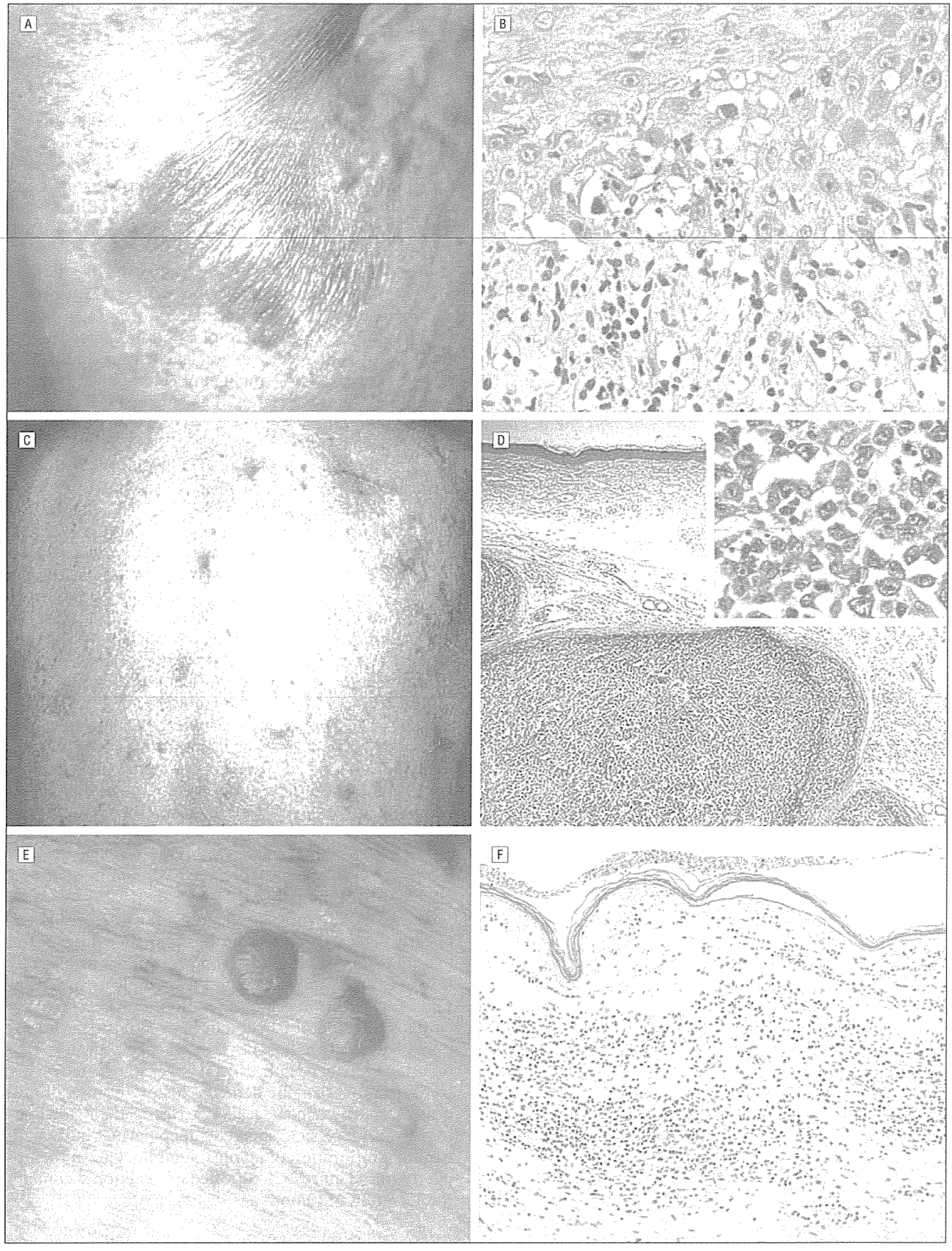

Figure 1. Case 1. A, Dark reddish macules on the trunk corresponding with typical mycosis fungoides. B, A bandlike upper dermal infiltrate with epidermotropism of small atypical lymphocytes (hematoxylin-eosin, original magnification ×400). C, Multiple nodules on her back. D, Tumor cells from nodule are located intradermally (hematoxylin-eosin, original magnification ×50). Atypical cells are large and have an abundant cytoplasm (hematoxylin-eosin, original magnification ×400) (inset). Those cells are CD30 positive. E, Blisters are seen on her right thigh. F, Subepidermal bullae are seen with atypical lymphocytes (hematoxylin-eosin, original magnification ×100).

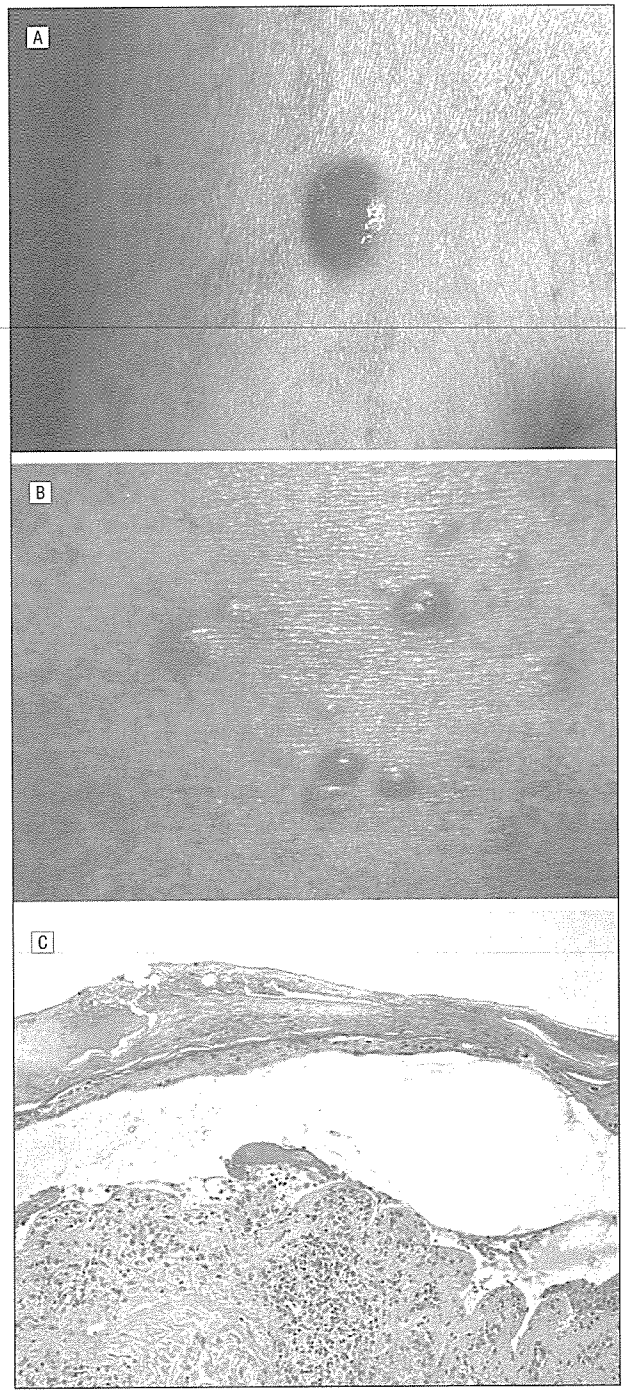

Figure 2. Case 2. A, Indurated tumor on her shoulder. B, Vesicles are scattered over erythrodermic skin. C, Subepidermal bullae are seen with atypical lymphocytes (hematoxylin-eosin, original magnification ×100).

lous lesions. Only 9 cases of vesiculobullous lesions with MF that have been reported have met these criteria.[1] The appearance of MFB in patients with MF is thought to indicate a poor prognosis. The patient in case 2 died within 2 months after the appearance of vesicles, which is a typical clinical course.[1]

The mechanism of blister formation has not been elucidated. Several hypotheses have been proposed: (1) Epidermotropism and accumulation of neoplastic cells in the basal layer of the epidermis may induce the loss of coherence between the basal lamina and the basal keratinocytes. (2) Cytokines released from neoplastic cells may intrude the normal connection between keratinocytes.

Transformed MF, defined as the presence of large cells exceeding 25% of the total lymphoid infiltrate in MF, is rare and associated with poor survival.[2,3] To our knowledge, case 1 is the first example of MFB occurring in a patient with T-MF. Both conditions are thought to be extremely rare and usually have limited survival; therefore, the coexistence of MFB and T-MF is thought to be a rare phenomenon. The relationship between blister formation and T-MF is unclear. While anaplastic large cells in T-MF could release cytokines to induce blistering, the subepidermal blisters in case 1 were observed with MF cells, not anaplastic large cells. Although rare, MFB is regarded as an important clinical subtype of MF.

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1. Bowman PH, Hogan DJ, Sanusi ID. Mycosis fungoides bullosa: report of a case and review of the literature. *J Am Acad Dermatol*. 2001;45:934-939.
2. Diamandidou E, Colome-Grimmer M, Fayad L, Duvic M, Kurzrock R. Trans formation of mycosis fungoides/Sezary syndrome: clinical characteristics and prognosis. *Blood*. 1998;92:1150-1159.
3. Vergier B, de Muret A, Beylot-Barry M, et al; French Study Group of Cutaneous Lymphomas. Transformation of mycosis fungoides: clinicopathological and prognostic features of 45 cases. *Blood*. 2000;95:2212-2218.

Cutaneous Blastomycosis: A Clue for Reassessing the Recent Diagnosis of Pulmonary Sarcoidosis

Blastomyces dermatitidis is an endemic fungus that frequently infects the skin. Herein, we present a case of disseminated blastomycosis initially misdiagnosed as pulmonary sarcoidosis and cutaneous squamous cell carcinoma.

Report of a Case. A 64-year-old man from New Brunswick, the eastern part of Canada, was referred to the dermatology department for a painless verrucous plaque (3.5×1.5 cm) on the helix of his left ear that evolved over the previous 5 months (**Figure 1**). Squamous cell carcinoma was suggested by initial biopsy findings, which showed pseudoepitheliomatous hyperplasia. On analysis of specimens from a second biopsy, multiple broad-based budding yeasts compatible with *B dermatitidis* were disclosed under special staining (**Figure 2**). The diagnosis of cutaneous blastomycosis was further confirmed by positive fungal cultures.

Chain Saw Blade Granuloma: Reaction to a Deeply Embedded Metal Fragment

We report a case of foreign body granuloma caused by a metal fragment from a chain saw. Since our patient had no history of injury and since several incisional biopsy specimens showed no foreign body, we had difficulty arriving at the diagnosis. Finally, pantomography revealed a small piece of metal and x-ray microanalysis of this piece was consistent with the edge of a chain saw. To our knowledge, this is the first case reported of foreign body granuloma caused by a chain saw blade fragment.

Report of a Case. A 72-year-old Japanese man presented with a 6-month history of a nontender, hard, subcutaneous nodule on his lower jaw (**Figure 1** A). A biopsy specimen of the nodule demonstrated granulomatous inflammation throughout the dermis with collagen fiber degeneration (Figure 1B). We initially suspected infectious granuloma, such as cutaneous tuberculosis, a deep fungal infection, or a malignant tumor. However, cultures for mycobacteria, fungi, and bacteria all yielded negative results and findings from polymerase chain reaction analysis were also negative for *Mycobacterium tuberculosis*. Pantomography, performed to rule out a dental fistula, revealed a foreign body in the lower jaw (**Figure 2**A). A piece of metal at a depth of about 7 mm was removed (Figure 2B). The piece was a 4.0×3.5-mm, sharp-edged shard of metal (Figure 2C). By x-ray microanalysis, it was found to be composed of tungsten, cobalt, argent, zinc, cadmium, and copper. We questioned the patient in detail whether he had had any exposure to metallic materials. His occupational history indicated that he had been a forestry worker and he used to operate chain saws. The piece of metal was consistent with the edge of a chain saw. Taken together, the metal fragment found from his lower jaw might have become inadvertently implanted in the skin during his work. The small piece of metal subsequently produced a large subcutaneous granulomatous allergic reaction.

Comment. Cobalt and zinc are among the metals capable of inducing granulomatous reactions.[1,2] The metal from ear piercing and eyebrow tattooing can produce allergic contact granuloma.[3,4] Although we could not precisely confirm which agent induced the granuloma in our patient, we suggest that the cause could be one of the metals that can induce allergic contact granuloma. This case presented several diagnostic difficulties because of the absence of a history of specific injury causing implantation of the metal. Other cases of metal implantation without a history of injury include graphite,[5] a broken piece of mower blade, and a wire stabbing. Therefore, foreign body granulomas should be included in the differential diagnoses of any granulomatous skin lesion even if there is no history of metal implantation. We recommend ra-

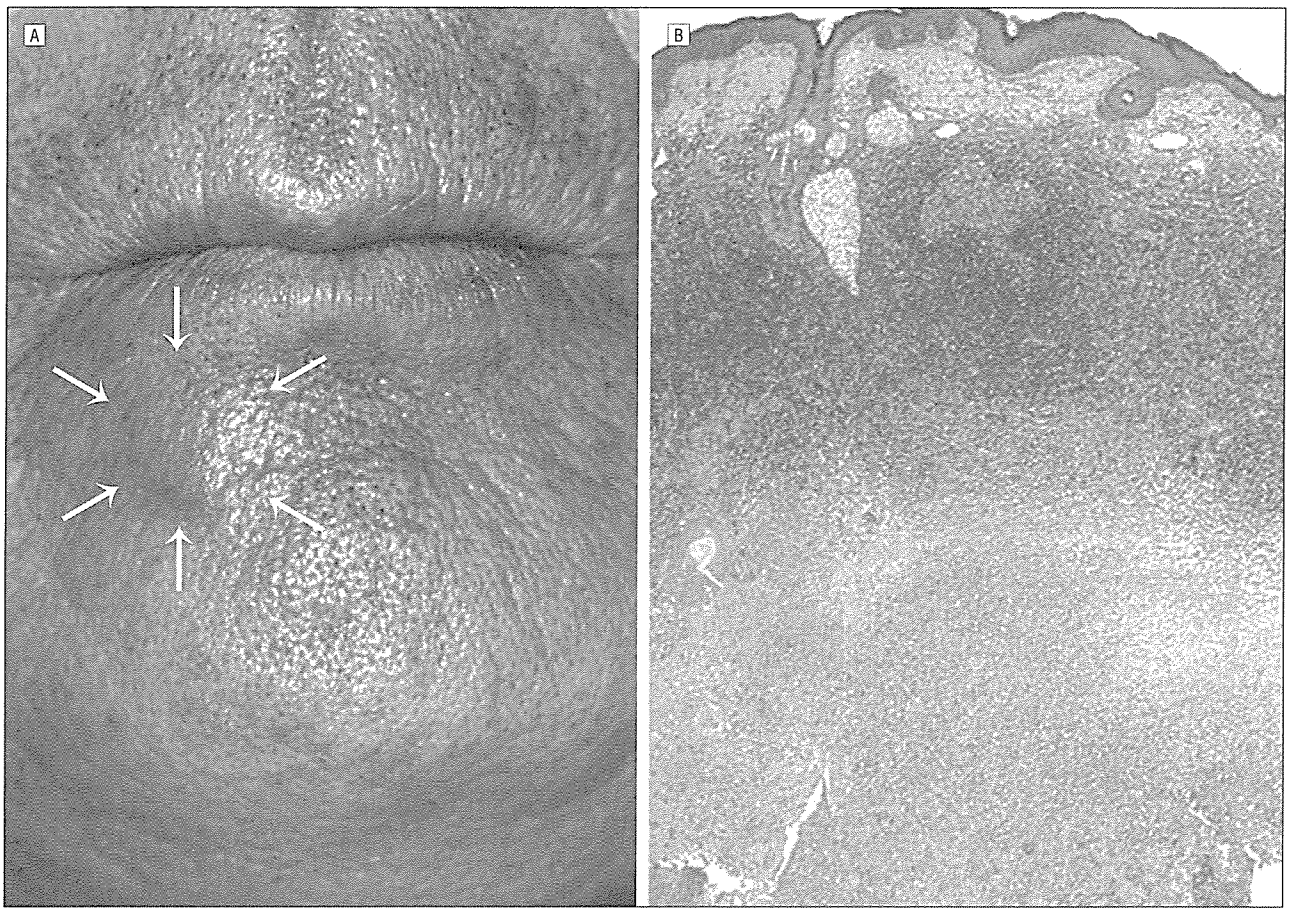

Figure 1. Clinical and histopathologic findings. A, A nontender, 2.5×2.0-cm, dark-reddish subcutaneous hard nodule on the lower jaw. B, Histopathologic examination showed granulomatous inflammation throughout the dermis with collagen fiber degeneration (hematoxylin-eosin, original magnification ×4).

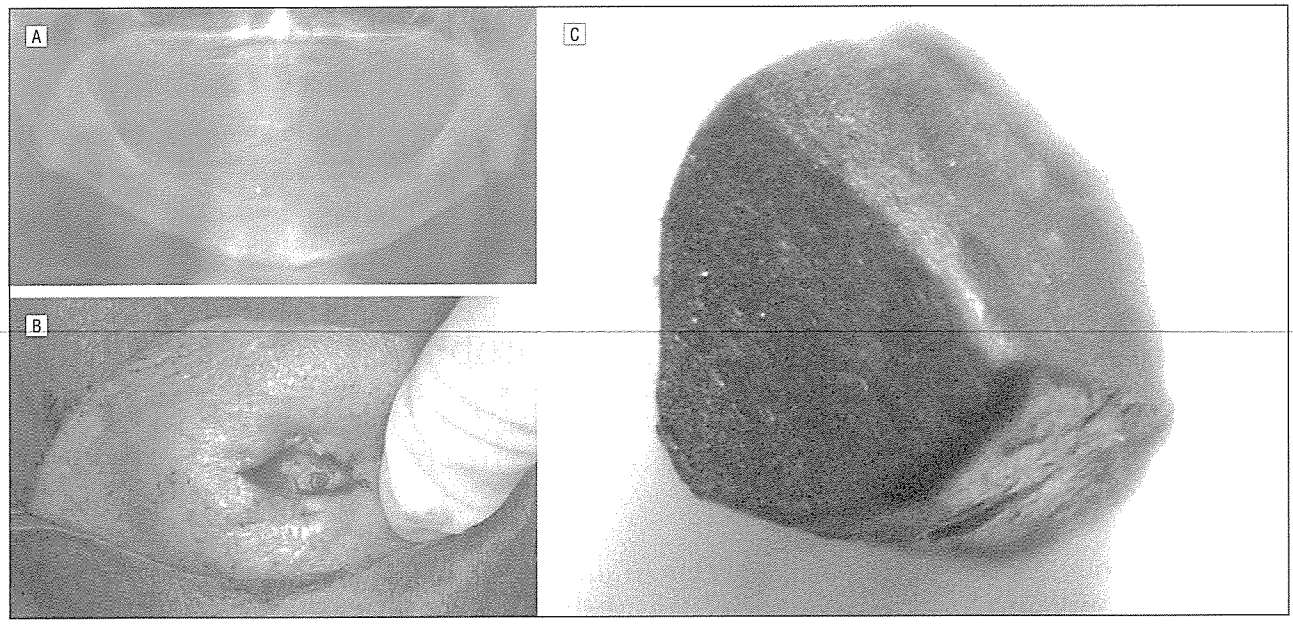

Figure 2. A, Pantomography revealed an electron-dense foreign body in the soft tissue of the patient's lower jaw. B, A piece of metal was seen within the lower dermis at a depth of about 7 mm. C, A 4.0×3.5-mm-diameter sharp-edged shard of metal (high-power view, orginal magnification ×10).

diographic imaging when a superficial biopsy specimen fails to show evidence of a deeply implanted foreign body.

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1. Jordaan HF, Sandler M. Zinc-induced granuloma: a unique complication of insulin therapy. *Clin Exp Dermatol.* 1989;14:227-229.
2. Pryce DW, King CM. Orofacial granulomatosis associated with delayed hypersensitivity to cobalt. *Clin Exp Dermatol.* 1990;15:384-386.
3. Ro YS, Lee CW. Granulomatous Tissue reaction following cosmetic eyebrow tattooing. *J Dermatol.* 1991;18:352-355.
4. Casper C, Groth W, Hunzelmann N. Sarcoidal-type allergic contact granuloma: a rare complication of ear piercing. *Am J Dermatopathol.* 2004;26:59-62.
5. Terasawa N, Kishimoto S, Kibe Y, Takenaka H, Yasuno H. Graphite foreign body granuloma. *Br J Dermatol.* 1999;141:774-776.

Acute Generalized Exanthematous Pustulosis Induced by Clindamycin

Acute generalized exanthematous pustulosis (AGEP) involves numerous nonfollicular sterile pustular lesions associated with fever above 38°C, neutrophilic leukocytosis, an intensely pruritic rash, and in later stages, desquamation.[1] A high proportion of cases are triggered by drugs, especially macrolides and aminopenicillins. We report the first case, to our knowledge, of AGEP associated with the use of clindamycin in a black woman who was also undergoing therapy for systemic lupus erythematosus (SLE).

Report of a Case. A 38-year old black woman with a history of SLE, hypertension, diabetes mellitus, and depression presented with a 9-day history of an increasingly widespread, painful, and pruritic eruption composed of erythematous macules and papules, which occurred 4 days after starting oral clindamycin hydrochloride therapy (300 mg, 3 times a day) for a suspected intravenous site infection during a hospital admission for SLE-related pleuritis (**Figure 1**). Her medications included prednisone, methotrexate, fluoxetine, valacyclovir hydrochloride, alendronate sodium, atenolol, losartan potassium, hydroxychloroquine sulfate, clonidine, amlodipine besylate, furosemide, and insulin. She denied the use of alcohol or any herbal or over-the-counter medications. The erythematous plaques were studded with flaccid 1- to 2-mm pustules and involved more than 80% of her body surface area. Lesions evolved to broad areas of desquamation. Target erythematous lesions favored the extensors and were not mucosal. A discrete grouping of pustules was noted in the buccal mucosa, posterior hard palate, and subungually. Palmar erythema on the thenar and hypothenar eminences was also present. There was no scalp scaling, nail pitting, or other stigmata of psoriasis.

Therapy with clindamycin was discontinued a few days after the eruption began. She denied exposure to any new drugs for several years and never had pustular or psoriatic lesions.

She had a white blood cell count of $22.6 \times 10^3/\mu L$, with 99% neutrophils. Findings from blood and urine cultures, taken both prior to the initiation of clindamycin therapy and during this admission, were negative. Histopathological examination revealed numerous neutrophils, eosinophils, and subcorneal pustules, supporting a diagnosis of AGEP (**Figure 2**).

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The Therapeutic Effects of Basic Fibroblast Growth Factor Contained in Gelatin Hydrogel Microspheres on Experimental Osteoarthritis in the Rabbit Knee

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Objective. **To investigate the therapeutic effects of basic fibroblast growth factor (bFGF) contained in gelatin hydrogel microspheres on osteoarthritis (OA) development in rabbit knee joints.**

Methods. **^{125}I-labeled bFGF contained in gelatin hydrogel microspheres was administered to the knee joints of normal rabbits to confirm the sustained-release kinetics of bFGF in the knee joint. In addition, the expression of proteoglycan core protein messenger RNA was examined using real-time polymerase chain reaction to confirm the anabolic effects on the cartilage treated with the sustained release of bFGF. The bFGF in gelatin hydrogel microspheres was administered to the knee joint once every 3 weeks (a total of twice) from 4 weeks after anterior cruciate ligament transection (ACLT). Ten weeks after ACLT, gross morphologic and histologic examinations were performed.**

Results. **Sustained release of bFGF in the knee joint continued for >7 days and induced the anabolic effects on the cartilage. Intraarticular injections of bFGF contained in gelatin hydrogel microspheres suppressed the progression of OA in the ACLT rabbit model.**

Conclusion. **Our findings demonstrated that sustained release of bFGF into the joint had therapeutic effects on OA development in a rabbit model. Our results suggest the potential feasibility of a new conservative treatment for OA.**

The pathology of osteoarthritis (OA) is based on degeneration of the extensive articular cartilage. Since joint function is remarkably affected, daily living and social activities of patients with OA are restricted. The articular cartilage, which is the matured hyaline cartilage, has no vessels, and the cells for repair cannot be provided. There are limits to the division potential of the cartilage cells and their ability to be repaired. Therefore, it becomes difficult to treat OA when it progresses and the articular cartilage degenerates.

As a conservative treatment for OA, intraarticular (IA) injection of a hyaluronan preparation has been used (1), but its efficacy on OA progression has limitations. It has been reported that orally administered supplements, such as glucosamine and chondroitin sulfate, reduce pain and inhibit narrowing of the joint space width in OA patients (2,3), and these supplements have attracted attention. However, these supplements cannot change the natural course of OA or greatly suppress the progression of OA. Currently, most cases of advanced OA cannot be treated using procedures such as autologous transplantation of articular cartilage (4,5) and cartilage cells (6), which are used for localized articular cartilage injury, and can only be managed by artificial joint replacements, but there are problems concerning the degree of invasion, cost, and long-term prognosis. For these reasons, an effective conservative treatment to inhibit OA progression is needed.

In a recent study, an attempt was made to regenerate the injured articular cartilage to the hyaline cartilage using various growth factors to accelerate car-

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tilage metabolism or inhibit cartilage degeneration (7). Among growth factors, basic fibroblast growth factor (bFGF) is regarded as one of the most potent mitogens for chondrocytes in vitro (8). In many studies, bFGF is applied in treatments for injured cartilage (7,9,10). Since the results of these studies show that bFGF is effective in localized articular cartilage injury, there is a possibility that IA injection of bFGF can inhibit OA progression by its beneficial effect on cartilage, although the pathology of OA and cartilage injury are different. However, it has been reported that when administered in vivo, bFGF is rapidly diffused from the injection site and metabolized (11,12).

When a large amount of protein is repeatedly injected into the joints, biologic effects of growth factors influence other components of the joint (e.g., subchondral bone and synovium), which may induce adverse events. In addition, bFGF shows both anabolic and catabolic actions in the articular cartilage, depending on its concentrations (13). Therefore, in the treatment of cartilage with bFGF it is important to control effective local concentrations. Development of a drug delivery system in which appropriate doses of a growth factor are slowly and continuously released into the joint for a certain period has been needed. We previously focused on gelatin hydrogel microspheres as a sustained-release system (14). When bFGF was impregnated with acid gelatin, bFGF was continuously released with decomposition of the gelatin in vivo. This gelatin could be reduced to particles and injected intraarticularly.

The purpose of this study was to investigate the efficacy of intraarticularly injected, controlled-release bFGF by gelatin hydrogel microspheres as a new approach to managing the progression of OA in a rabbit model of OA.

MATERIALS AND METHODS

Preparation of gelatin hydrogel microspheres. Gelatin hydrogel microspheres were prepared by glutaraldehyde crosslinking of a gelatin aqueous solution dispersed in an oil phase, as previously described (15). Gelatin with an isoelectric point of 4.9 (Nitta Gelatin, Osaka, Japan), isolated from bovine bone collagen using an alkaline process, was used. The water content of the gelatin hydrogel microspheres used in this study was 95%. The average diameter of the microspheres was 70 μm, with a range from 45 to 75 μm.

Impregnation of bFGF into gelatin hydrogel microspheres. An aqueous solution of human recombinant bFGF with an isoelectric point of 9.6 (10 mg/ml) was purchased from Kaken (Tokyo, Japan). The bFGF was impregnated into the gelatin microspheres by dropping 10 mg/ml or 1 mg/ml of bFGF solution (10 μl) onto 1 mg of freeze-dried gelatin microspheres, which were then maintained at room temperature for 1 hour. The solution was completely absorbed into the microspheres during swelling, since the solution volume was less than that theoretically required for the equilibrated swelling of microspheres.

Preparation of animals. Japanese white rabbits (2 kg) (Oriental BioService, Kyoto, Japan) were used. Before the experiments, animals were anesthetized with intramuscular injections of xylazine (10 mg) (Bayer, Tokyo, Japan) and ketamine (50 mg) (Sankyo, Tokyo, Japan). The hair around the knee joints of the lower extremities was shaved, and the sites were disinfected with povidone iodine. This study was conducted according to the guidelines for animal research of the Kyoto Prefectural University of Medicine.

Kinetic analysis of bFGF release in the rabbit knee joint. An in vivo release test for bFGF in the knee joints of rabbits was performed using ^{125}I-labeled bFGF (16). Na^{125}I (74 MBq/100 μl) was purchased from PerkinElmer (Boston, MA). The bFGF was radioiodinated with chloramine T according to the method described by Greenwood et al (17). Ten microliters of labeled bFGF solution (37940.5 counts per minute/μl) was impregnated into the prepared gelatin microspheres (1 mg), as described above.

The ^{125}I-labeled bFGF contained in gelatin hydrogel microspheres (labeled bFGF microspheres) was administered to the left knee joint and a solution form of labeled bFGF (labeled bFGF solution) was administered to the right knee joint of the rabbits (n = 9). Rabbits were killed 1, 3, and 7 days after administration of the bFGF (n = 3 per day), and the persistent radioactivity in the knee joints was measured using a Cobra II gamma counter (Packard, Meriden, CT).

Real-time polymerase chain reaction (PCR) analysis of messenger RNA (mRNA) of proteoglycan core protein and type II collagen (CII). Gelatin hydrogel microspheres containing 100 μg bFGF in 300 μl phosphate buffered saline (PBS) (100 μg bFGF microspheres) were injected into the left knee joint of the rabbits (n = 10). Solution (100 μg bFGF solution) was also injected into the left knee joint of the rabbits (n = 10). Rabbits were killed 1, 3, 7, 14, and 21 days after administration (n = 2 per day), and the cartilage from both knees (right side as control) was collected. Tissues were stored in RNAlater (Qiagen, Hilden, Germany). The total RNA was isolated from the cartilage using an RNeasy Minikit (Qiagen), in which cartilage was homogenized with a microhomogenizer (New Generation Instrument Technique, Chiba, Japan) in Buffer RLT for 2 minutes (18). The ratio of the optical density at 260 nm to the optical density at 280 nm of the isolated total RNA was >1.7. Single-stranded complementary DNA (cDNA) synthesis was performed for each RNA sample using an RNA PCR kit (Applied Biosystems, Foster City, CA). Random hexamers were used to prime cDNA synthesis.

Quantitative real-time PCR was performed with a Biosystem 7300 sequencer (Applied Biosystems) by monitoring the increase in the reporter fluorescence of a TaqMan probe for the proteoglycan core protein or CII during PCR. The PCR primers and TaqMan probe were designed by Assays-by-Design Service (Applied Biosystems). In a 25-μl PCR, 1 μl of cDNA (100 ng) was amplified, using 50 n*M* forward and reverse primers, a 200 n*M* ribosomal RNA (rRNA) probe (VIC-TAMRA labeled), and TaqMan 2× Universal PCR Master Mix (Applied Biosystems) for the internal control; 20×

mix primers, TaqMan probe (FAM-TAMRA labeled), and TaqMan 2× Universal PCR Master Mix for the target gene. Thermal cycling was performed using 40 cycles of 95°C for 15 seconds, followed by 60°C for 1 minute. The gene-specific primers and TaqMan probe were as follows: for proteoglycan core protein, forward 5′-CGCCTACCAGGACAAGGT-3′, reverse 5′-GCGCAGGCTCTGGATCTC-3′, and probe 5′-[FAM]TCGCTGCCCAACTAC[TAMRA]-3′; and for CII, forward 5′-CCTGTGCGACGACATAATCTGT-3′, reverse 5′-GCAGTGGCGAGGTCAGTAG-3′, and probe 5′-[FAM] CAGTCCTTGGTGTCTTC[TAMRA]-3′.

For quantification of the changes in gene expression, we used the comparative C_t method to calculate the relative fold changes normalized against the rRNA (19). Values are the means of 3 samples, and each sample was assayed in duplicate. Gene expression was quantified as the difference between the C_t value of the sample for the target gene and the mean C_t value of that sample for the endogenous control (ΔC_t) (rRNA). Relative expression was calculated as the difference between the C_t values of the test sample and the control sample ($\Delta\Delta C_t$). Relative expression of genes of interest was calculated and expressed as $2^{-\Delta\Delta C_t}$.

Administration of gelatin hydrogel microspheres into the knee joint in the rabbit OA model. OA was induced in the left knee joint of Japanese white rabbits by performing unilateral transection of the anterior cruciate ligament (ACLT) (20). Postoperatively, ACLT rabbits were permitted activity in a 35 × 38 × 53–cm cage. The rabbits were closely monitored for infections and other complications.

Fifty-seven ACLT rabbits were divided into 6 groups. The first group underwent no injection after ACLT (control group; n = 15). The second group (PBS in microspheres [PBS-M]; n = 7) had 1 mg of gelatin hydrogel microspheres containing PBS injected into the ACLT knee. As solution groups, the third and fourth groups were injected with 10 μg bFGF (10-bFGF–S; n = 6) and 100 μg bFGF (100-bFGF–S; n = 5) in solution, respectively. As treatment groups, the fifth (10 μg bFGF in microspheres [10-bFGF–M]; n = 11) and sixth (100 μg bFGF in microspheres [100-bFGF–M]; n = 13) groups received IA injections of 1 mg gelatin hydrogel microspheres containing 10 μg and 100 μg of bFGF, respectively. Each injection was performed twice (4 and 7 weeks after ACLT).

Assessment of OA. *Gross morphologic examination.* Gross morphologic changes of the femoral condyles were evaluated 10 weeks after ACLT. Findings were classified by 6 grades (grade 1 = intact articular surface; grade 2 = minimal fibrillation; grade 3 = overt fibrillation; grade 4a = erosion of 0–2 mm; grade 4b = erosion of 2–5 mm; and grade 4c = erosion of >5 mm) using India ink, and the grades were scored from 0 to 5 (20). Three orthopedic surgeons (HT, KS, and MS) evaluated samples independently, and grades were decided by agreement among 2 or more surgeons.

Histologic examination. Ten weeks after ACLT, the femoral condyles and patella were fixed in 3.7% formaldehyde for 10 days and decalcified with formic acid. After decalcification, the femoral condyles were cut along the sagittal plane, and both condyles were embedded in paraffin. Three sections were prepared from the lateral condyle, where the most grossly apparent changes appeared, and 1 section of synovium surrounding the patella was prepared. Sections (3 μm thick) were cut and stained with hematoxylin and eosin and with Safranin O. The cartilage was scored from 0 to 14 using the Mankin scale (21).

Statistical analysis. All data are reported as the mean ± SEM. Student's *t*-test was used to compare the ratios of the remaining bFGF in the knee joints of the rabbits. Nonparametric Mann-Whitney U test was used to evaluate the statistical significance of differences in the gross morphologic OA score and the Mankin scores. *P* values less than 0.05 were considered significant.

RESULTS

Controlled release of bFGF in the knee joints of rabbits. Residual radiation doses were measured with a gamma counter 1, 3, and 7 days after administration of bFGF. Mean ± SEM residual radiation doses 1 and 3 days after bFGF administration were 9.1 ± 3.5% and 2.4 ± 1.4%, respectively, in rabbits administered labeled bFGF-S, and 46.4 ± 5.2% and 11.7 ± 2.8%, respectively, in those administered labeled bFGF-M. The amount of labeled bFGF that remained in the knee joint cavity was significantly higher in rabbits administered labeled bFGF-M than in those administered labeled bFGF-S ($P < 0.05$; n = 3).

These results indicated that gelatin hydrogel microspheres slowly released bFGF into the knee joint cavity. In rabbits administered labeled bFGF-M, the residual radiation dose 7 days after administration decreased, but was still 3.4 ± 0.78%. This showed that bFGF administered to the knee joint cavity remained for 7 days (Figure 1). Labeled bFGF-M was localized in the soft tissue, including the synovium, meniscus, and ante-

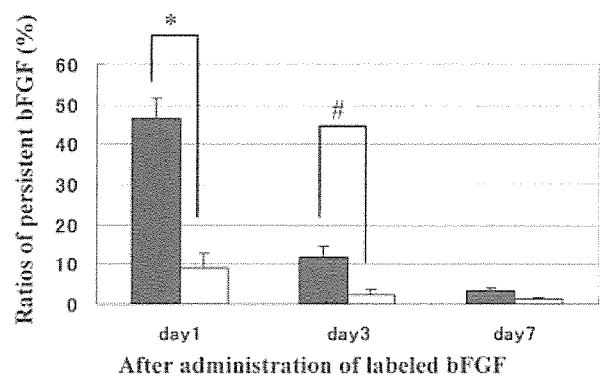

Figure 1. Kinetics of basic fibroblast growth factor (bFGF) release from gelatin hydrogel microspheres. Labeled bFGF contained in gelatin hydrogel microspheres or a solution of labeled bFGF was administered to rabbit knees. Persistent radioactivity in the knee joint was measured using a gamma counter. Solid bars represent the ratio of the remaining radioactivity in the knee joint injected with labeled bFGF microspheres. Open bars represent the ratio of the remaining radioactivity in the knee joint injected with labeled bFGF solution. Values are the mean and SEM. * = $P < 0.01$; # = $P < 0.05$.

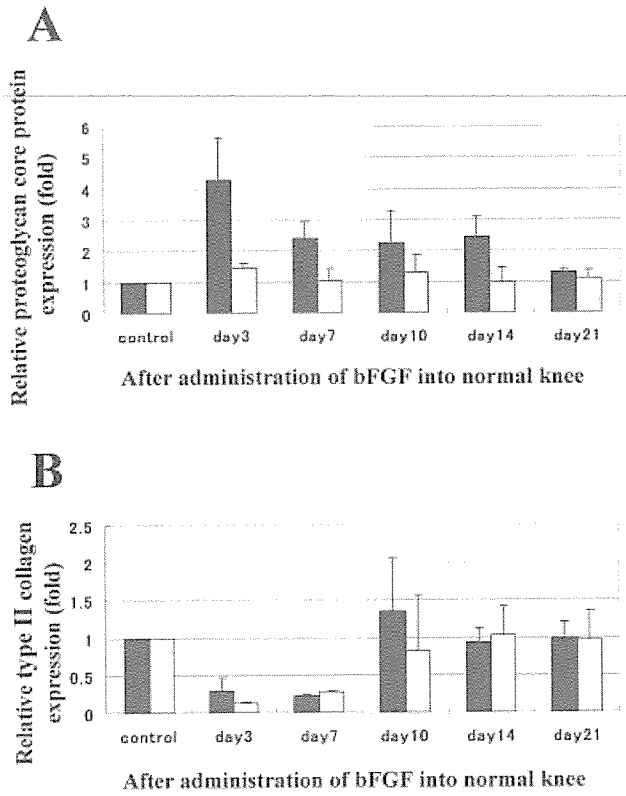

Figure 2. Anabolic effects on extracellular matrix gene expression by the sustained release of basic fibroblast growth factor (bFGF) in vivo. Gelatin hydrogel microspheres containing 100 μg bFGF or 100 μg bFGF solution were injected into the left knee joint. The time course of relative expression of mRNA for **A,** proteoglycan core protein and **B,** type II collagen was examined by real-time polymerase chain reaction. Solid bars represent the relative expression of mRNA in the cartilage of the knee joint injected with 100 μg bFGF microspheres. Open bars represent the relative expression of mRNA in the cartilage of the knee joint injected with 100 μg bFGF solution. Values are the mean and SEM.

rior and posterior cruciate ligaments, but not in the articular cartilage (data not shown).

In vivo anabolic effects of sustained release of bFGF on extracellular matrix (ECM) gene expression. Proteoglycan core protein mRNA was increased 4.3-, 2.4-, 2.3-, 2.4-, and 1.3-fold on days 3, 7, 10, 14, and 21, respectively, after administration of bFGF in cartilage from knee joints treated with 100 μg bFGF-M as compared with control cartilage (Figure 2A). There was no anabolic change in the cartilage treated with bFGF-S. This indicated that the sustained release of bFGF into the joint accelerated metabolism of the articular cartilage. In contrast, expression of CII mRNA was suppressed for 7 days after administration of 100 μg bFGF-M (Figure 2B).

Therapeutic effects on OA development. *Gross morphology.* Gelatin hydrogel microspheres containing bFGF were administered to the left knee joint once every 3 weeks (a total of twice) from 4 weeks after ACLT. Ten weeks after ACLT, the left knee joint was harvested and examined macroscopically and histologically. Macroscopically, severe OA of grade 4a or higher was observed in 60% of the control group, 43% of the PBS-M group, 50% of the 10-bFGF–S group, 40% of the 100-bFGF–S group, 18% of the 10-bFGF–M group, and 15% of the 100-bFGF–M group.

Severe OA markedly decreased in the 100-bFGF–M and 10-bFGF–M groups (Figure 3A). The

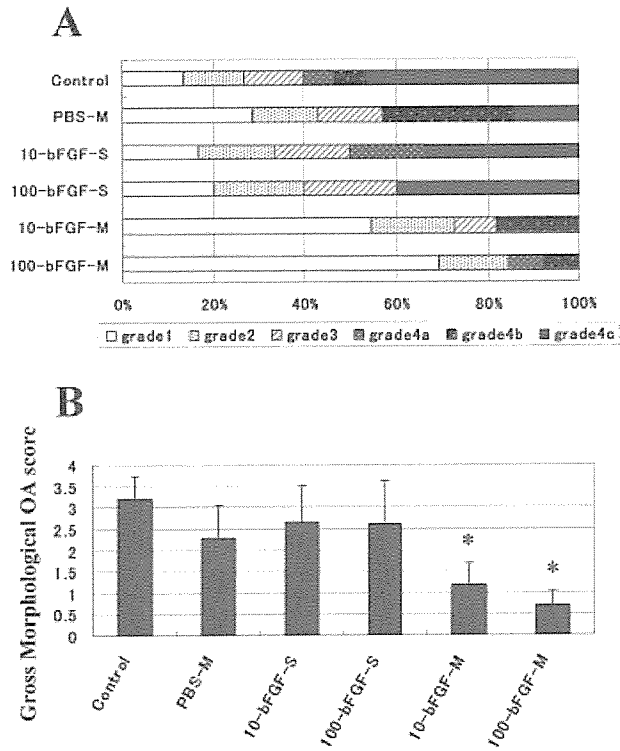

Figure 3. Therapeutic effects of basic fibroblast growth factor (bFGF) microspheres (bFGF-M) evaluated by gross morphologic examination. Fifty-seven male Japanese white rabbits underwent anterior cruciate ligament transection (ACLT) of the left knee and were divided into 6 groups. All bFGF injections were administered 4 and 7 weeks after ACLT. Gross morphologic examinations were performed 10 weeks after ACLT. **A,** Gross morphologic grading of osteoarthritis (OA). Grade 1 = intact articular surface; grade 2 = minimal fibrillation; grade 3 = overt fibrillation; grade 4a = erosion of 0–2 mm; grade 4b = erosion of 2–5 mm; grade 4c = erosion of >5 mm. **B,** Gross morphologic OA score (range 0–5). Values are the mean and SEM. PBS-M = phosphate buffered saline microspheres; 10-bFGF–S = 10 μg bFGF solution; 100-bFGF–S = 100 μg bFGF-S; 10-bFGF-M = 10 μg bFGF microspheres; 100-bFGF–M = 100 μg bFGF-M; * = $P <$ 0.05 versus control group.

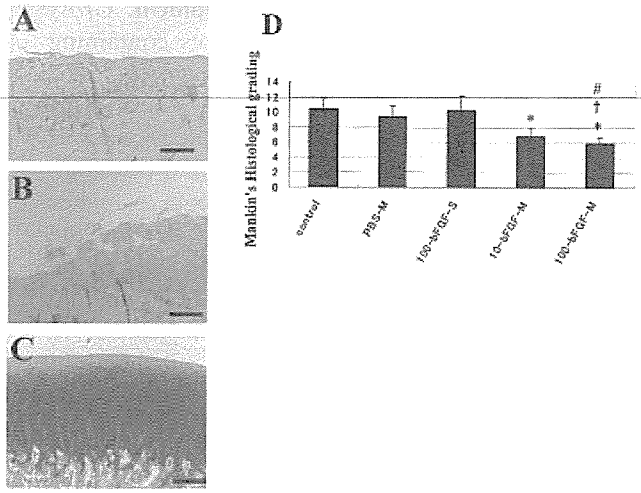

Figure 4. Therapeutic effects of bFGF-M evaluated by histologic examination 10 weeks after ACLT. Sagittal lateral femoral condyles of the sections were stained with Safranin O. Shown are representative microscopic images of knees from rabbits in the groups administered **A,** PBS-M, **B,** 100-bFGF–S, and **C,** 100-bFGF–M. (Original magnification × 200; bars = 150 μm.) **D,** Mean and SEM OA grades (Mankin scale) in tissues from control (n = 10), PBS-M (n = 7), 100-bFGF–S (n = 5), 10-bFGF–M (n = 11), and 100-bFGF–M (n = 13) groups 10 weeks after ACLT. * = $P < 0.05$ versus control group; † = $P < 0.05$ versus PBS-M group; # = $P < 0.05$ versus 100-bFGF–S group. See Figure 3 for definitions.

mean ± SEM gross morphologic OA score was 1.18 ± 0.50 in the 10-bFGF–M group, 0.70 ± 0.36 in the 100-bFGF–M group, 3.20 ± 0.52 in the control group, and 2.29 ± 0.78 in the PBS-M group which indicates that the severity of OA in the treatment groups of bFGF-M was significantly less than that in the control and PBS-M groups ($P < 0.05$). The gross morphologic OA score in the 10-bFGF–S and 100-bFGF–S groups was 2.67 ± 0.84 and 2.60 ± 1.03, respectively, which showed that the severity of OA in the treatment groups was less than that in the bFGF-S group (Figure 3B). No clear formation of bone spurs was observed macroscopically in either group (data not shown). These findings suggest that IA injections of 100-bFGF–M and 10-bFGF–M suppressed the progression of OA in an ACLT model.

Histology. In grade 4 tissue from the lateral condyle of the femur in the PBS-M and 100-bFGF–S groups, the surface layer of the articular cartilage was irregular, and the intensity of Safranin O staining in the matrix was decreased. The laminar structure of the articular cartilage was destroyed, and cluster formation was observed in the cartilage cells (Figures 4A and B). These findings were observed in all tissue that was macroscopically confirmed to be grade 4. In contrast, in grade 1 tissue from the 100-bFGF–M group, the intensity of Safranin O staining decreased in some parts of the surface layer of the articular cartilage, but the surface of the articular cartilage was generally smooth, and the laminar structure was maintained (Figure 4C). According to the Mankin scale, the severity of OA in the bFGF-M treatment groups was significantly less than that in the control group ($P < 0.05$) (Figure 4D).

Histologic observation of grade 4 synovium from the control and PBS-M groups showed proliferation of the synovial cells, infiltration of the inflammatory cells, and vascularity. Infiltration of the inflammatory cells and vascularity were also observed in grade 4 joints from the 100-bFGF–M group, but no remarkable differences compared with the control or PBS-M group were found (results not shown). This result suggested that inflammation of the synovium was not due to administration of bFGF or gelatin hydrogel microspheres, but was due to OA changes.

DISCUSSION

The ACLT rabbit model of OA has been well characterized, and is reported to be useful for investigations of OA (20). This model shows mild OA changes 4 weeks after ACLT, and grade 4 OA (full thickness of ulceration) in ~60% of the joints 9 weeks after ACLT. Hyaluronan, which is currently used for OA treatment in clinical settings, is reported to decrease grade 4 OA by ~40% in this model when it is injected intraarticularly once a week from 4 to 8 weeks after ACLT, with evaluation at 9 weeks after ACLT (22,23).

In our study, rabbits were killed 10 weeks after ACLT to measure OA, and severe OA of grade 4 or higher was observed in 60% of the control group. These results prove the consistency and reproducibility of this OA model. In contrast, grade 4 OA was observed in 15–18% of the rabbits administered bFGF contained in gelatin hydrogel microspheres, which indicates that this drug strongly suppresses the progression of OA. This effect was not observed with the intact bFGF solution. Therefore, it might be important to maintain the bFGF concentrations by using a sustained-release system of gelatin hydrogel microspheres. This is the first study to show that a sustained-release formulation of a growth factor suppresses the progression of OA in an animal model.

Endothelial cells and mesenchymal cells produce bFGF in vivo, which then binds to the ECM. In the wound-healing process, the matrix is degraded by enzymes, and bFGF is released and acts to induce neovas-

cularization and tissue regeneration (24). The effect of bFGF cannot be maintained, even when it is locally injected, because its biologic half-life is short (11,12). Therefore, methods such as continuously infusing bFGF into the joints with an osmotic pump (25,26) and introducing bFGF genes into the cells with a virus vector (27) have been used in studies to treat animals with localized articular cartilage injuries.

Since these methods require the complicated techniques involved with virus vectors and safety must be maintained, it is currently difficult to apply them to clinical practice. When bFGF is impregnated with acid gelatin, an ionic bond is formed and bFGF is continuously released with the degradation of the gelatin. The release rate depends not on the amount of impregnated bFGF, but on the rate of gelatin degradation (i.e., the percentage of water in the gelatin) (28). In our system, since the sustained release of a growth factor depends on the degradation of a carrier, it was possible to slowly release the growth factor, irrespective of its size or configuration. This system uses easy techniques and can be applied via IA injections.

It has been shown that bFGF is a potent mitogen for a wide variety of cell types derived in vitro from the mesoderm and neuroectoderm (29). It accelerates the proliferation and differentiation of cartilage cells (8) and stabilizes their phenotype (7). It also promotes matrix synthesis of cartilage cells (10). When the effect of bFGF on cartilage injuries is suppressed by using a neutralization antibody, cartilage repair is suppressed. Therefore, bFGF is thought to be a key factor in cartilage repair (25). In our study, expression of proteoglycan core protein mRNA was greatly increased for 14 days by the administration of bFGF in microspheres. Actual levels of increased matrix production were not examined in our study. However, there was a similar tendency in the change of proteoglycan core protein mRNA and of ^{35}S sulfate uptake (30,31). Our results suggest that bFGF in microspheres accelerates anabolism in cartilage metabolism.

The mechanism of OA suppression by bFGF in microspheres in this animal model is unknown, but the promotion of cartilage matrix metabolism by bFGF might play an important role. Inflammatory cytokines (e.g., interleukin-1 and tumor necrosis factor α) and reactive oxygen species induced by them (e.g., protease and nitric oxide) are deeply involved in the progression of OA (32). Aggrecanases are also important for OA progression. It has recently been reported that a key enzyme of cartilage degeneration in OA is ADAMTS-5 (aggrecanase 2) (33). To clarify the mechanism of action of bFGF in microspheres, it might be necessary to investigate their influence on those factors.

The optimal treatment protocol for the use of bFGF in microspheres in rabbits is uncertain. The suppressive effect on OA progression was observed even when bFGF in microspheres was administered once every 3 weeks. Moreover, in the ACLT knee, the release kinetics of bFGF in microspheres may be shorter than in the normal knee due to the influence of overexpression of proteolytic enzymes, such as matrix metalloproteinases. Therefore, OA progression could be more favorably suppressed when the administration interval is decreased. Chuma et al (36) used an osmotic pump system to continuously infuse bFGF into rabbit joints, and reported that 1-day treatment has the same ability to repair the cartilage as 2-week treatment.

In this study, the expression of proteoglycan core protein mRNA in the articular cartilage increased for 14 days after administration of bFGF in microspheres, while bFGF that remained in the joint 3 and 7 days after administration decreased to ~11.7% and 3.4%, respectively. There is a possibility that bFGF is effective for protective and repair actions in the articular cartilage even when its IA concentrations are not maintained for a long period. However, this effect was not observed when bFGF was administered as a simple solution in this study. Therefore, the maintenance of IA bFGF concentrations for a certain period might be necessary.

Sah et al (13) report that low concentrations of bFGF accelerate the production of glycosaminoglycan and inhibit catabolism, and high concentrations of bFGF suppress the production of glycosaminoglycan and increase the release of glycosaminoglycan from the cartilage. The actions of bFGF on the articular cartilage are complicated. In future studies, ideal bFGF administration intervals and the optimal bFGF concentration released from gelatin hydrogel microspheres for the suppression of OA progression should be determined.

In this study, the inhibitory effects of IA injections of bFGF in microspheres on the progression of OA were revealed. However, its regenerative properties against advanced OA are not clear. A sustained-release bFGF formulation could show greater efficacy in combination with other techniques, such as cell therapies. In future studies, the mechanism of the suppression of OA progression by IA injection of bFGF in microspheres should be clarified and regeneration treatment for degenerated cartilage should be further developed. The results of the present study, however, demonstrate that IA injection of bFGF in microspheres could be a useful conservative treatments for OA in the future.

REFERENCES

1. Jubb RW, Piva S, Beinat L, Dacre J, Gishen P. A one-year, randomised, placebo (saline) controlled clinical trial of 500-730 kDa sodium hyaluronate (Hyalgan) on the radiological change in osteoarthritis of the knee. Int J Clin Pract 2003;57:467–74.
2. Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, et al. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. Lancet 2001;357:251–6.
3. Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. Arch Intern Med 2002;162:2113–23.
4. Yamashita F, Sakakida K, Suzu F, Takai S. The transplantation of an autogeneic osteochondral fragment for osteochondritis dissecans of the knee. Clin Orthop Relat Res 1985;201:43–50.
5. Hangody L, Kish G, Karpati Z, Udvarhelyi I, Szigeti I, Bely M. Mosaicplasty for the treatment of articular cartilage defects: application in clinical practice. Orthopedics 1998;21:751–6.
6. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331: 889–95.
7. Weisser J, Rahfoth B, Timmermann A, Aigner T, Brauer R, von der Mark K. Role of growth factors in rabbit articular cartilage repair by chondrocytes in agarose. Osteoarthritis Cartilage 2001;9 Suppl A:S48–54.
8. Kato Y, Iwamoto M, Koike T. Fibroblast growth factor stimulates colony formation of differentiated chondrocytes in soft agar. J Cell Physiol 1987;133:491–8.
9. Yamamoto T, Wakitani S, Imoto K, Hattori T, Nakaya H, Saito M, et al. Fibroblast growth factor-2 promotes the repair of partial thickness defects of articular cartilage in immature rabbits but not in mature rabbits. Osteoarthritis Cartilage 2004;12:636–41.
10. Fujimoto E, Ochi M, Kato Y, Mochizuki Y, Sumen Y, Ikuta Y. Beneficial effect of basic fibroblast growth factor on the repair of full-thickness defects in rabbit articular cartilage. Arch Orthop Trauma Surg 1999;119:139–45.
11. Kawaguchi H, Kurokawa T, Hanada K, Hiyama Y, Tamura M, Ogata E, et al. Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rats. Endocrinology 1994;135:774–81.
12. Shida J, Jingushi S, Izumi T, Iwaki A, Sugioka Y. Basic fibroblast growth factor stimulates articular cartilage enlargement in young rats in vivo. J Orthop Res 1996;14:265–72.
13. Sah RL, Chen AC, Grodzinsky AJ, Trippel SB. Differential effects of bFGF and IGF-I on matrix metabolism in calf and adult bovine cartilage explants. Arch Biochem Biophys 1994;308:137–47.
14. Tabata Y, Nagano A, Ikada Y. Biodegradation of hydrogel carrier incorporating fibroblast growth factor. Tissue Eng 1999;5:127–38.
15. Ikada Y, Tabata Y. Protein release from gelatin matrices. Adv Drug Deliv Rev 1998;31:287–301.
16. Yamamoto M, Tabata Y, Ikada Y. Ectopic bone formation induced by biodegradable hydrogels incorporating bone morphogenetic protein. J Biomater Sci Polym Ed 1998;9:439–58.
17. Greenwood FC, Hunter WM, Glover JS. The preparation of I-131-labelled human growth hormone of high specific radioactivity. Biochem J 1963;89:114–23.
18. Gehrsitz A, McKenna LA, Soder S, Kirchner T, Aigner T. Isolation of RNA from small human articular cartilage specimens allows quantification of mRNA expression levels in local articular cartilage defects. J Orthop Res 2001;19:478–81.
19. Bustin SA. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems [review]. J Mol Endocrinol 2002;29:23–39.
20. Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. Osteoarthritis Cartilage 1996;4:87–98.
21. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.
22. Yoshioka M, Shimizu C, Harwood FL, Coutts RD, Amiel D. The effects of hyaluronan during the development of osteoarthritis. Osteoarthritis Cartilage 1997;5:251–60.
23. Shimizu C, Yoshioka M, Coutts RD, Harwood FL, Kubo T, Hirasawa Y, et al. Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. Osteoarthritis Cartilage 1998;6:1–9.
24. Taipale J, Keski-Oja J. Growth factors in the extracellular matrix [review]. FASEB J 1997;11:51–9.
25. Cuevas P, Burgos J, Baird A. Basic fibroblast growth factor (FGF) promotes cartilage repair in vivo. Biochem Biophys Res Commun 1988;156:611–8.
26. Hiraki Y, Shukunami C, Iyama K, Mizuta H. Differentiation of chondrogenic precursor cells during the regeneration of articular cartilage. Osteoarthritis Cartilage 2001;9 Suppl A:S102–8.
27. Yokoo N, Saito T, Uesugi M, Kobayashi N, Xin KQ, Okuda K, et al. Repair of articular cartilage defect by autologous transplantation of basic fibroblast growth factor gene–transduced chondrocytes with adeno-associated virus vector. Arthritis Rheum 2005;52: 164–70.
28. Tabata Y. Tissue regeneration based on growth factor release [review]. Tissue Eng 2003;9 Suppl 1:S5–15.
29. Gospodarowicz D, Neufeld G, Schweigerer L. Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells [review]. Cell Differ 1986;19:1–17.
30. Cs-Szabo G, Melching LI, Roughley PJ, Glant TT. Changes in messenger RNA and protein levels of proteoglycans and link protein in human osteoarthritic cartilage samples. Arthritis Rheum 1997;40:1037–45.
31. Takahashi K, Kubo T, Arai Y, Kitajima I, Takigawa M, Imanishi J, et al. Hydrostatic pressure induces expression of interleukin 6 and tumour necrosis factor α mRNAs in a chondrocyte-like cell line. Ann Rheum Dis 1998;57:231–6.
32. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. Arthritis Rheum 1998;41: 1266–74.
33. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 2005;434:644–8.
34. Hongbin W, Jingyuan D, Linyun C, Yuming D. Carboxymethylated chitin reduces MMP-1 expression in rabbit ACLT osteoarthritic cartilage. Ann Rheum Dis 2004;63:369–72.
35. Takahashi K, Goomer RS, Harwood F, Kubo T, Hirasawa Y, Amiel D. The effects of hyaluronan on matrix metalloproteinase-3 (MMP-3), interleukin-1β (IL-1β), and tissue inhibitor of metalloproteinase-1 (TIMP-1) gene expression during the development of osteoarthritis. Osteoarthritis Cartilage 1999;7:182–90.
36. Chuma H, Mizuta H, Kudo S, Takagi K, Hiraki Y. One day exposure to FGF-2 was sufficient for the regenerative repair of full-thickness defects of articular cartilage in rabbits. Osteoarthritis Cartilage 2004;12:834–42.