Localized scleroderma associated with *Borrelia burgdorferi* infection

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**Background:** Recent reports have implicated *Borrelia burgdorferi* infection as a possible cause of localized scleroderma (LS).

**Objective:** Our purpose was to describe the clinical, histologic, and immunopathologic features of patients with LS who had serum antibodies to *B. burgdorferi*.

**Methods:** Ten patients were examined clinically and by routine microscopy. Biopsy specimens from seven patients were studied immunohistochemically with monoclonal antibodies. The proliferative response of peripheral blood mononuclear cells to *B. burgdorferi* was investigated in seven patients by lymphocyte proliferation assay.

**Results:** Seven patients had plaque-type morphea, and three patients had linear scleroderma. Two patients had a history of previous erythema migrans. One patient had coexistent acrodermatitis chronica atrophicans, and in two patients lichen sclerosus et atrophicus was observed. Histologically, a prominent inflammatory phase with sclerosis of the connective tissue was shown in all patients. Immunohistochemical studies revealed that the inflammatory infiltrates consisted of both B and T lymphocytes, predominantly of the CD4+ subset. All 10 patients had strongly elevated serum antibodies to *B. burgdorferi*. Patients with LS showed significantly elevated lymphoproliferative responses to *B. burgdorferi* when compared with healthy control subjects.

**Conclusion:** Our findings suggest that some cases of LS are linked to *Borrelia* infection.

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Localized scleroderma (morphea) is characterized by circumscribed sclerotic plaques but also may occur as a linear band or as guttate lesions. An infectious cause has long been suspected because improvement in early inflammatory lesions may occur after antibiotic therapy.

Since *Borrelia burgdorferi* was discovered to be the cause of erythema chronicum migrans (ECM), acrodermatitis chronica atrophicans (ACA), and lymphadenosis benigna cutis (LBC), many reports have implicated *B. burgdorferi* spirochetes as the cause of morphea.

We report the clinical and histologic features of 10 patients with localized scleroderma in whom *B. burgdorferi* infection was suggested on the basis of elevated antiborrelial antibodies. To gain more insight into the role of cellular immune mechanisms in the pathogenesis of scleroderma associated with *B. burgdorferi* infection, we performed an immunophenotyping of the dermal inflammatory infiltrate with a panel of monoclonal antibodies. In addition, T-cell immune responses to *B. burgdorferi* were studied with a lymphocyte proliferation assay.

**PATIENTS AND METHODS**

**Patients**

Ten patients with a clinical diagnosis of localized scleroderma were studied. The patients were selected because of typical clinical findings and antibodies to *B. burgdorferi*. The patients' histories were reviewed for duration of the disease, antecedent tick bites, location of lesions, and types of associated skin disease.

In all patients, skin biopsy specimens were obtained.
All tissue specimens were fixed in formalin and were stained with hematoxylin and eosin and van Gieson. In addition, biopsy specimens from seven patients were bisected and one half was immediately frozen in liquid nitrogen and used for immunohistochemical studies. Heparinized peripheral blood samples were obtained from seven patients at the time of the first examination and the proliferative responses of mononuclear cells to *B. burgdorferi* were studied.

**Immunohistochemical staining**

Frozen sections (6 μm) were cut on a cryostat, mounted on gelatin-coated slides, and fixed in acetone at 4°C for 10 minutes. The sections were incubated with monoclonal antibodies to anti-Leu-4/CD3 (pan-T cells), anti-Leu-3a/CD4 (helper/inducer T cells), anti-Leu-2a/CD8 (suppressor/cytotoxic T cells), anti-Leu-6/CD1 (Langerhans cells), anti-Leu-14/CD22 (B cells), anti-Leu-11b/CD16 (natural killer cells), anti-IL-2/CD25 (interleukin 2 receptor), and anti-HLA-DR (Becton Dickinson, Mountain View, Calif.) The immunoperoxidase staining procedure was performed as described previously. The sections were counterstained with hematoxylin and examined by standard light microscopy.

**Lymphocytic proliferative assay**

Lymphocyte proliferation assays were performed as previously described according to the method of Weir. Mononuclear cells (PBMCs) were isolated from heparinized peripheral venous blood by Histopaque-1077 (Sigma, Dorset, U.K.) density-gradient centrifugation. The lymphocyte-rich layer was removed from the interface, washed with Hank's balanced salt solution, and PBMCs were adjusted to a final concentration of 1 x 10^6 cells/ml and resuspended in culture medium 199 (Gibco BRL, Grand Island, N.Y.) containing 15% human AB serum. Cells (2 x 10^5) were added in triplicate to each well of tissue culture plate and stimulated by 20 μl of sonicated *B. burgdorferi* isolate B31. Control wells received medium only. Cells were cultured at 37°C in a humidified atmosphere containing 5% carbon dioxide for 4 days. Lymphocyte proliferation was measured by the incorporation of tritiated thymidine (Amersham Int. Ltd., Bucks, U.K.). During the final 18 hours of culture, 0.5 mCi of tritiated thymidine was added to each well. Cells were harvested with a semiautomated collector and the incorporated radioactivity was measured with a liquid scintillation counter (TRI-CARB 1500, Packard). Proliferation was expressed as the mean counts per minute (cpm) for triplicate culture, and stimulation indices (SI) were calculated (mean cpm with stimulant/mean cpm without stimulant). Twenty-one healthy volunteers with no history or symptoms of *Borrelia* infection served as controls. All control subjects were seronegative for *B. burgdorferi* antibodies. The results were examined statistically by means of an unpaired *t* test.

**CASE REPORT**

A 47-year-old man had a 6-month history of slowly expanding indurated skin lesions on his right arm. He had had tick bites on several occasions in an area known to be infested by *Ixodes ricinus* that were infected with *B. burgdorferi*. A year before ECM had developed and had been treated with oral penicillin, 2 million units daily, for 12 days. Examination revealed a linear sclerotic hypopigmented band on the dorsal right arm extending from the elbow to the shoulder (Fig. 1). In addition, the patient had a circumscribed, slightly atrophic, pigmented, nonindurated plaque on his right anterior chest and small plaques of lichen sclerosus et atrophicus (LSA) on his neck. Laboratory investigations revealed a positive immunofluorescent antibody test to *B. burgdorferi* with an IgG titer higher than 1:160. The patient had a vigorous lymphocyte proliferative response to *B. burgdorferi* with a stimulation index of 20.7. The patient was treated with oral doxycycline, 200 mg daily, for 2 weeks. Two months after the completion of therapy the sclerotic lesions have become less indurated, but there was only minimal improvement of the atrophic lesions.

**RESULTS**

**Clinical observations**

Ten patients, five men and five women, 16 to 77 years of age, were seen with clinical evidence of localized scleroderma. Their clinical features are summarized in Table I. Three patients were able to recall a tick bite, and two had well-documented ECM. Skin lesions developed 3 months to 2 years before the patients were seen. Five patients had sol-
Table I. Clinical features, serum antibodies and lymphoproliferative responses to *Borrelia burgdorferi* in 10 patients with localized scleroderma

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at diagnosis (yr)/Sex</th>
<th>Clinical type</th>
<th>Sites of involvement</th>
<th>Duration of disease (mo)</th>
<th>Associated skin diseases</th>
<th><em>B. burgdorferi</em> antibodies (IFA)</th>
<th>Lymphoproliferative responses to <em>B. burgdorferi</em> (stimulation index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47/M</td>
<td>Linear scleroderma</td>
<td>Upper arm</td>
<td>12</td>
<td>LSA, APP</td>
<td>++ &gt;1:160</td>
<td>20.7</td>
</tr>
<tr>
<td>2</td>
<td>52/M</td>
<td>Linear scleroderma</td>
<td>Arm</td>
<td>3</td>
<td></td>
<td>— &gt;1:160</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>16/F</td>
<td>Linear scleroderma + morphea</td>
<td>Left arm, chest</td>
<td>6</td>
<td></td>
<td>+ &gt;1:160</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>28/F</td>
<td>Plaque morphea</td>
<td>Left leg</td>
<td>12</td>
<td></td>
<td>— &gt;1:160</td>
<td>8.8</td>
</tr>
<tr>
<td>5</td>
<td>43/M</td>
<td>Plaque morphea</td>
<td>Back</td>
<td>4</td>
<td>APP</td>
<td>— &gt;1:160</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>47/F</td>
<td>Plaque morphea</td>
<td>Thigh</td>
<td>24</td>
<td></td>
<td>— &gt;1:160</td>
<td>n.d.</td>
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<tr>
<td>7</td>
<td>56/F</td>
<td>Plaque morphea</td>
<td>Left leg</td>
<td>24</td>
<td></td>
<td>— &gt;1:160</td>
<td>n.d.</td>
</tr>
<tr>
<td>8</td>
<td>34/M</td>
<td>Plaque morphea</td>
<td>Thigh</td>
<td>12</td>
<td></td>
<td>— &gt;1:160</td>
<td>8.3</td>
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<tr>
<td>9</td>
<td>72/F</td>
<td>Multiple plaques of morphea</td>
<td>Leg</td>
<td>6</td>
<td>LSA</td>
<td>— &gt;1:160</td>
<td>8.8</td>
</tr>
<tr>
<td>10</td>
<td>77/M</td>
<td>Multiple plaques of morphea</td>
<td>Legs</td>
<td>3</td>
<td>ACA</td>
<td>— &gt;1:160</td>
<td>9.2</td>
</tr>
</tbody>
</table>

ACA, Acrodermatitis chronica atrophicans; APP, atrophoderma Pasini-Pierini; LSA, lichen sclerosus et atrophicus; n.d., not done.

itary plaque morphea. In four of them the lesion was present on a lower extremity; one had localized scleroderma on the back. The plaques were inflamed and edematous or surrounded by a violaceous halo. Two patients had multiple lesions of morphea on the lower extremities. Three patients had linear scleroderma; one had a band of induration with hypopigmentation on the arm, whereas in the other two deep bandlike erythematous and edematous skin changes with a coarse peau d'orange appearance were observed on the forearms (Fig. 2). One of the latter two patients had typical morphea on the chest 3 months later. One patient had ACA on the leg, and in one patient atrophoderma was observed. One patient had typical lesions of LSA, and another patient had LSA and atrophoderma simultaneously.

**Histologic findings**

Light microscopic examination of biopsy specimens taken from seven patients with solitary and multiple plaques of morphea showed varying degrees of sclerosis and hyalinization of the connective tissue. The epidermis was atrophic in two patients and showed acanthosis with slight hyperkeratosis in another two. There was a perivascular and interstitial cellular infiltrate extending from the papillary dermis into the underlying subcutis in four patients; this was limited to the papillary and mid dermis in three. The infiltrate consisted of both lymphocytes and plasma cells. Large numbers of perivascular and interstitial plasma cells were found in three patients and were seen occasionally in another three. Nodules of lymphocytes and plasma cells were present at the junction of the reticular dermis and subcutis with focal accumulations of plasma cells within the subcutaneous tissue in two patients. Biopsy specimens from patients with linear scleroderma revealed an extensive hyaline sclerosis of connective tissue involving the entire dermis in one patient and swelling and homogenization with mild sclerosis of collagen in another two. This was associated with a prominent perivascular and interstitial infiltrate consisting of lymphocytes and numerous plasma cells diffusely through the papillary and reticular dermis and extending into the subcutis. In one patient with early and severe inflammation circumscribed areas of closely packed, thick collagen bundles, numerous fibroblasts, and a marked interstitial lymphoplasmacytic infiltrate were observed in the reticular dermis (Fig. 3). In 3 of 10 patients a focal hydropic degeneration of the basal cell layer of the epidermis was observed. Two biopsy specimens from atrophic lesions (cases 1 and 9) were consistent with LSA.

**Immunohistochemical findings**

The immunophenotype of the inflammatory infiltrate was studied in seven patients. In all cases, the majority of the dermal infiltrate was strongly reactive with pan-T cell anti-CD3 antibodies. Among
these T cells the number of CD4+ cells outnumbered CD8+ cells. CD8+ cells were predominant in one biopsy specimen. Small numbers of CD8+ cells associated with focal lymphocytic liquefaction of the basal cell layer were present in three cases. T cells of CD4+ and CD8+ subsets were observed between the collagen bundles in close apposition to the fibroblasts (Fig. 4). The class II molecule HLA-DR was expressed by almost all dermal infiltrate cells and fibroblasts. Compared with normal skin, the number of CD1+ Langerhans cells was increased, mainly in the lower epidermis in three cases. A few Langerhans cells were also present within the dermal infiltrate.

In the deep reticular dermis, there were patchy collections of CD22+ B cells in four of seven specimens. IL-2 receptor expression was found on a few cells in the dermal infiltrate. The dermal infiltrate showed negativity for natural killer cell antigen as recognized by the anti-CD16 antibody.

**Serologic findings**

The indirect immunofluorescence assay was used for the detection of both IgM and IgG antibodies to *B. burgdorferi*. All 10 patients had an elevated IgG titer of more than 1:160. IgM antibody was found in three patients.

**Lymphoproliferative responses to *B. burgdorferi***

The lymphoproliferative response to *B. burgdorferi* was studied in seven patients. The mean *B. burgdorferi*-induced lymphocyte response of patients with localized scleroderma (4881 ± 3249 cpm; range 2640 to 11,924 cpm) was higher than that of the healthy control subjects (1705 ± 889 cpm; range 568 to 3743 cpm).

The mean SI of patients was 9.5 ± 5.3 and ranged from 5.1 to 20.7. In contrast, the 21 healthy control subjects had SIs that ranged from 1.0 to 7.5 with a mean of 3.5 ± 2.2 (*p* < 0.0001).

**DISCUSSION**

Localized scleroderma usually appears for no known reason, although it has been reported to be related to trauma, infection, or immunologic injury.1-3 Recently, infection with *B. burgdorferi* has been suggested as a cause.7 With both indirect immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA) elevated serum antibody...
to *B. burgdorferi* has been found in 20% to 50% of patients with morphea, compared with 5% to 14% in controls. In contrast, several studies have found no increase in antibody frequency or level in similar patients with morphea. This contradiction may be caused by the lack of standardization of current serologic methods and the use of different spirochetal strains. Aberer et al. showed the presence of spirochetes in histologic sections from three of eight patients who had morphea with an antibody against *B. burgdorferi* and an avidin-biotin immunoperoxidase method. In addition, spirochetes were cultured from one patient’s biopsy specimen taken from the lilac halo of morphea. Weber et al. reported the successful cultivation of spirochetes from lesions of two seronegative patients with morphea. Spirochete-like organisms in lesions of morphea have also been detected by silver-stained sections of skin. ACA, clearly a disorder caused by *B. burgdorferi* infection, has been reported with coexisting morphea. In addition, sclerodermatous lesions and lesions similar to LSA or morphea may occur within the lesions of ACA in about 10% of patients. Some authors have postulated that these represent ACA with sclerosis rather than idiopathic scleroderma.

However, other investigators have reported that typical plaque-type morphea may develop independent of preexisting ACA. In addition, Büchner reported the case of a patient with ECM in whom morphea developed at the site of an initial ECM lesion that resolved after antibiotic treatment. Two of our patients had a history of previous ECM, and, in one patient with plaques of morphea, ACA was found on a lower extremity. The association of morphea with ECM or ACA is reasonable clinical confirmation of a relationship. The coexistence of typical LSA with morphea in two of our patients suggests that these lesions are part of a continuous disease spectrum and may represent different manifestations of *Borrelia* infection. According to this concept, ACA has been reported to coexist with LSA in several patients. Because atrophoderma of Pasini and Pierini is considered to be an atrophic variant of morphea, it is not surprising that changes of this type were observed in this study.

Three of our patients had linear scleroderma. In two the lesions resembled eosinophilic fasciitis, although there was no history of exertion or trauma and no blood eosinophilia. Nevertheless, recent reports suggested *B. burgdorferi* infection as the cause of eosinophilic fasciitis based on the presence of antibodies against *Borrelia* and the demonstration of spirochetes in tissue. Histologically, all our patients showed a prominent inflammatory phase with varying degrees of sclerosis and hyalinization of the connective tissue. The inflammation can be more intense in the papillary dermis and can extend from the dermis into the underlying subcutis. Lymphocytes and plasma cells formed the predominant cell type at all levels of the dermis and lymphoid aggregates with plasma cells were also observed at the level of the subcutis. A striking histologic finding was the presence of lymphocytes and plasma cells in close apposition to and within the collagen bundles throughout the dermis. Our immunohistochemical studies showed that the inflam-
matory infiltrates consisted of both B and T lymphocytes, predominantly of the CD4+ subset, which were in close contact with HLA-DR+ fibroblasts. In agreement with a recent report by Aberer et al., CD1+ dendritic Langerhans cells were significantly increased in the epidermis in our study.

The presence of large numbers of CD1+ Langerhans cells suggests that they may be involved in the inflammatory process by presenting B. burgdorferi antigen to the CD4+ T cells. In addition, the intense expression of HLA-DR antigens indicates that most of the T cells and fibroblasts are in an activated state. Several investigators reported positive T-cell proliferative responses to B. burgdorferi in seropositive and seronegative patients with Lyme borreliosis, as determined by lymphocyte proliferation assay. However, elevated B. burgdorferi–induced lymphoproliferative responses have been also described in patients with other diseases and in healthy control subjects. Thus false-positive responses can occur, presumably because many persons are sensitized to cross-reactive or equivalent antigens in other organisms. However, in our patients the overall mean lymphocyte response was higher than the response in controls. This may indicate some degree of immune specificity. Bühner et al. also reported markedly elevated lymphoproliferative responses to B. burgdorferi in 11 of 28 patients (39%) with localized scleroderma. Breier et al. found heightened lymphocytic responses to whole B. burgdorferi in 45% of seropositive and 21% of seronegative patients with morphea. Cellular immune responses decreased after antibiotic treatment. Recently, Yssel et al. reported that B. burgdorferi selectively activates a subset of human CD4+ T cells with a specific pattern of lymphokine secretion in Lyme arthritis. There is also evidence that macrophages stimulated by B. burgdorferi produce large amounts of IL-1. The continuous production of various lymphokines such as IL-1 by B. burgdorferi–reactive T cells may be a mechanism by which the spirochetes initiate the fibrotic process by influencing fibroblast metabolism.

REFERENCES
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