The expanding spectrum of cutaneous borreliosis

K. EISENLE, B. ZELGER

The known spectrum of skin manifestations in cutaneous Lyme disease is continuously expanding and can not be regarded as completed. Besides the classical manifestations of cutaneous borreliosis like erythema (chronicum) migrans, borrelial lymphocytoma and acrodermatitis chronica atrophicans evidence is growing that at least in part also other skin manifestations, especially morphea, lichen sclerosus and cases of cutaneous B-cell lymphoma are causally related to infections with Borrelia. Also granuloma annulare and interstitial granulomatous dermatitis might be partly caused by Borrelia burgdorferi or similar strains. There are also single reports of other skin manifestations to be associated with borrelial infections like cutaneous sarcoidosis, necrobiosis lipoidica and necrobiotic xanthogranuloma. In addition, as the modern chameleon of dermatology, cutaneous borreliosis, especially borrelial lymphocytoma, mimics other skin conditions, as has been shown for erythema anulare centrifugum or lymphocytic infiltration (Jessner Kanof) of the skin.

KEY WORDS: Borrelia infections - Lymphoma, B-cell - Granuloma annulare - Immunohistochemistry - Lyme disease - Necrobiosis lipoidica.

The germ Borrelia (B.) burgdorferi is a slowly growing microaerophil gram negative spirochete. The generation time is about 7-12 hours. At the time 13 different species are included in the B. burgdorferi sensu latu (s.l.) complex. They show different geographic distributions and are associated with different vectors and hosts. For example B. garinii and B. afzelii are frequently found in Europe but not in the United States. The different species of Borrelia also show different patterns of pathogenicity. Only B. burgdorferi sensu strictu, B. garinii, B. afzelii and B. spielmanii are clearly known to cause disease in the human host. Different Borrelia species also show a different tissue preference in humans (tissue tropism). While B. burgdorferi sensu strictu mainly affects the joints, B. garinii shows a preference for the nervous system, while B. afzelii most frequently affects the skin. The pathogenicity for other species like B. valaisiana, B. lusitaniae und B. lonestari is not definitely clear. All the other species, like B. bisetii, B. japonica or B. californiensis seem not to cause disease in the human host. In addition there are a few atypical and not yet classified strains of B. burgdorferi s.l. in Europe and in the USA.

Overview about the diseases caused by B. burgdorferi

Lyme disease is the most frequent tick born disease in the northern hemisphere. Borrelia are mainly transmitted in Europe by Ixodes (I.) ricinus, in Asia by I. persulcatus and in the United States by I. scapularis.

Abbreviation list.—ACA: acrodermatitis chronica atrophicans; BL: borrelial lymphocytoma; EM: erythema migrans; FFM: focus floating microscopy; LS: lichen sclerosus

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I. pacificus or Amblyomma americanum, also named “lone star tick” for the prominent white dot on the back of the adult female. Borrelia cause characteristic diseases and, similar to syphilis, borreliosis has been separated into three stages. Stage I (stage of first manifestation) comprises the erythema migrans (EM, Figure 1A) and the early borrelial lymphocytoma (B, Figure 2A), which develop weeks to months after the tick bite and are accompanied by mild influenza like symptoms. The second stage (stage of dissemination) includes the involvement of the musculoskeletal system (acute Lyme arthritis with additional painful muscles, tendons, bursae and bones), the nervous system (meningitis, lymphocytic meningoradiculoneuritis, also called Bannwarth syndrome, mild encephalitis and myelitis), the heart (atrioventricular block, myopericarditis, pancarditis), the skin with late borrelial lymphocytoma, acute inflammatory acrodermatitis chronica atrophicans (ACA Figure 3A) and multiple erythema migrans, as well as the involvement of all other organs (lymphadenopathy, splenomegaly, hepatitis, mild haematuria, conjunctivitis, iritis and ophthalmitis). The third stage (“stage of chronicity”) is limited to organ diseases with irreversible organic or functional damage in joints, nervous system or in the skin. This are in the case of skin diseases chronic-atrophic stages of ACA and in part morphea (Figure 4A) and lichen sclerosus. These chronic changes develop after months to years. Single stages might overlap or be completely missing.
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History of cutaneous borreliosis

In the newer literature infections with *B. burgdorferi sensu lato* are named after the city Lyme in Connecticut although first descriptions have occurred long before in Europe. Already Afzelius wrote in 1909 in the *Archiv für Dermatologie und Syphilis* about an EM caused by the tick *Ixodes reduvius* (*Ixodes scapularis* in the new taxonomy) and recognized already the tick as vector for this disease. The first description and nomination of ACA occurred even earlier in the year 1902 by Herxheimer and Hartmann. The BL was first described as *lymphadenosis cutis benigna* by Brävferstedt in 1943, Brävferstedt also suspected insect bites, mainly tick bites, as triggers of the disease. Finally, Willy Burdorfer discovered the spirochetal etiology of borreliosis (Lyme disease) in 1982 and the pathogenic agent was named *B. burgdorferi* in his honor.

Histopathologic patterns in classical cutaneous borreliosis

The dermatohistopathological changes in cutaneous borreliosis are a consequence of the continuous antigenic stimulus by persisting *Borrelia* in the tissue. The consequence are histopathologic similarities in between the different cutaneous manifestations, like structural changes in the collagen texture and the frequent presence of B lymphocytes, especially plasma cells. B lymphocytes can be easily shown by staining with anti-CD20 antibodies. The different clinical and histological manifestations can be explained by the location and duration of the infection and by the different known borrelial strains, on the other hand also by the dominating immune response involving B and T cells, as well as the number of inoculated bacteria and the genetic predisposition of the infected host (HLA type, disposition to autoimmune reactions). The damage in the connective tissue collagen is on one hand the result of the inflammatory infiltrate (“cytokine storm”, collateral damage), on the other hand *Borrelia* show collagenotropism and might directly influence and damage the collagen structure.

Histopathology of classical cutaneous borrelioses is characteristic, but unfortunately non specific, meaning that the diagnosis is first of all a clinical one. In the case of EM histopathology shows perivascular lymphocytic infiltrations not always containing plasma cells in all the dermal layers, sometimes with admixture of eosinophilic granulocytes and macrophages, as well as slight changes in the structure of the collagen texture in the connective tissue (Figure 1 B,C). In the case of ACA (Figure 3 B,C), depending on the duration of the disease, the changes include more or less pronounced atrophy of the epidermis, dermis and subcutaneous tissue with ectatic capillaries in the upper corium. The inflammatory infiltrate is accentuated perivascular or even band-like and contains plasma cells. In addition there is a loss of elastic fibers with the development of fibrosis or incipient sclerosis of the papillary and reticular dermis. Some authors consider BL as special form of an EM. Histopathology shows an unremarkable epidermis with sharp defined partially confluent lymphocytic infiltrates (Figure 2 B,C). There are two histopathologic types of BL with (follicular type) or without (diffuse/nodular type) follicular structures, resembling the germinal centers of lymph nodes. Combinations between the two types can be observed. Plasma cells and sometimes eosinophils and multinucleated giant cells can additionally be found at the border of the infiltrate. An unaltered grenz zone between the epidermis and the lymphocytic infiltrate can regularly be observed. Table I shows the expression of leukocyte differentiation antigens based on a score of 0-3+ in lesional skin.

Figure 4.—A) Late inflammatory poor “burned out” morphea; B, C) histopathology showing nearly absence of inflammatory infiltrates, atrophy of epidermis and marked sclerosis (H&E, b x10, c x100).
Diagnostic aids for cutaneous borreliosis

After initial enthusiasm, the detection of microorganisms has turned out to be difficult, frequently unreliable, and almost always extremely time-consuming by different procedures, including histochemical stains (Gram, Wright, Wright-Giemsa, and polychromes), fluorochromes (thioflavine-T, acridine orange, and rhodamine), silver impregnation techniques (Warthin-Starry, modified Dieterle, modified microwave-Dieterle, and Bosma-Steiner) in the 1980s, and immuno-histochemical analysis in the 1990s. Sero logic techniques (immunofluorescence, enzyme linked immunosorbent assay [ELISA], and immunoblot) are similarly unsatisfying, with false-negative (20-80%) and false positive results occasionally due to cross-reactions with Treponema pallidum or, more commonly, to a positive endemic background of 20% to 30% in many parts of Europe. Cultures with specified media such as modified Pettenkofer-Kelly or Barbour-Stoenner-Kelly can detect Borrelia in all clinical forms, but these techniques are limited to special laboratories and are unreliable, with less than 50% sensitivity. Moreover the time delay to get a positive culture can be up to four weeks. Molecular techniques initially seemed to solve the riddle, but in due course, it became clear that sensitivity varies (30-90%) according to the Borrelial strains, the material (fresh frozen tissue or paraffin material), and the applied primers. There is further a risk of contamination leading to false positive results. So, cutaneous borreliosis remains a diagnosis based on circumstantial evidence combining clinicopathologic and laboratory information and clinical response to therapy.

Immunohistochemistry and focus floating microscopy

The histopathologic diagnosis was recently made easier by the direct detection of the pathogen by immunohistochemistry and focus floating microscopy (FFM). The method is an advancement to older immunohistochemistry techniques employing a polyclonal anti-borrelial antibody, which recognizes all different borreliol strains. FFM combines several strategies to detect minuscule organisms in tissue sections. The key point to this technique is an almost holoscopic approach to the slide by tuning the focus of the microscope through the thickness of the slide (3-4 µm). So with FFM the section is scanned through in two planes: horizontally in serpentines as in routine cytology, and, simultaneously, vertically at a magnification of 200 to 400 times. This approach allows detection of B. burgdorferi (diameter 0.2 µm compared to 2 µm of collagen bundles) which pass through the section at various angles and accordingly may appear as undulated, comma-like to dot forms (Figure 5). In addition omission of counter stain as well as bright illumination of the scanning field proves to be helpful as the bright red color of the 3-amino-9-ethylcarbazole-stained microorganisms best contrasts with the faint yellow color of unstained collagen bundles as well as other tissue structures. The technique can be applied successfully on fresh material, nitrogen-frozen material and paraf-

### Table I

Expression of leukocyte differentiation antigens based on a score of 0-3+ in lesional skin of patients with various manifestations of dermatoborrelioses (EM erythema migrans, BL borrelial lymphocytoma, ACA acrodermatitis chronica atrophicans).19

<table>
<thead>
<tr>
<th>Score: 0-3+</th>
<th>CD68+ T cells</th>
<th>CD3+ T cells</th>
<th>CD4+ T cells</th>
<th>CD8+ T cells</th>
<th>CD20+</th>
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</thead>
<tbody>
<tr>
<td>EM (N=12)</td>
<td>++</td>
<td>++</td>
<td>+/-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>BL (N=5)</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>ACA (N=10)</td>
<td>++</td>
<td>++</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Figure 5.—Immunohistochemistry for Borrelia. Four photomicrographs of the same area focusing through the thickness of this section (focus floating microscopy). Note the (A) absence to varying appearance (B-D) of a cluster of Borrelia (Acris BP 1002, no counterstain, x1000).

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fin-embedded material and it is an easy, quick and inexpensive method to reliably detect *Borrelia* in cutaneous tissue sections.

FFM proved to be more sensitive than PCR- and ELISA-PCR in cases of classical borreliosis. Cases of prominent *Borrelia* detection by FFM are usually positive by PCR, which becomes negative in later stages as the number of microorganisms drop. So, it was possible to detect *Borrelia* by FFM in 47 of 71 ticks, in 34 of 66 tick bites, in 30 of 32 cases of EM, in 41 of 43 cases of BL (Figure 5) and in 50 of 51 cases of ACA. With a sensitivity of over 90% FFM was more sensitive than PCR with a sensitivity of 45% (*P*<0.001) and nearly equally specific (99% versus 100%). All control cases, except one case of false-positive secondary syphilis, were negative with FFM (Table II).

### Detection of *B. burgdorferi* in morphea

Morphea is an inflammatory connective tissue disorder of unknown etiology. The involvement of *B. burgdorferi* as a causative agent was first proposed by Aberer *et al.* in 1985. Since then conflicting results have been obtained by different studies using serological, immunohistochemical, culture and PCR approaches. *Borrelia* has been frequently detected in Europe and Asia patients, but not in cases from the United States or Scotland. Studies reporting a positive association between *B. burgdorferi* infection and morphea found evidence of the organism in 26-100% of cases (Table III); on the other hand there are at least 10 reports where no positive cases could be found. Such a variability in the detection rates can be explained by a combination of factors, including the clinical expression of morphea, the quality and amount of tissue used, and the sensitivity of the detection method used. A comprehensive review of the literature on the detection of *Borrelia* in morphea is provided in Table III.

### Table II.—Detection of *Borrelia* in classical cutaneous Lyme disease by focus floating microscopy (FFM) only and by direct comparison of FFM with PCR.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>FFM only</th>
<th>FFM PCR</th>
</tr>
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<tbody>
<tr>
<td>Ticks</td>
<td>47/71 [66.2]</td>
<td>NA NA</td>
</tr>
<tr>
<td>Tick bites</td>
<td>34/66 [51.5]</td>
<td>NA NA</td>
</tr>
<tr>
<td>Erythema chronicum migrans</td>
<td>15/17 [88.2]</td>
<td>15/15 [100]</td>
</tr>
<tr>
<td>Borrelial lymphocytoma</td>
<td>22/24 [91.7]</td>
<td>19/19 [100]</td>
</tr>
<tr>
<td>Acrodermatitis chronica atrophicans</td>
<td>22/23 [95.7]</td>
<td>28/28 [100]</td>
</tr>
<tr>
<td>Controls**</td>
<td>0/109 [0]</td>
<td>1/60 [1.7] **</td>
</tr>
</tbody>
</table>

**Total number of cases** 310 122 128

NA (not available). **Including atopic and stasis dermatitis, prurigo, insect bite, scabies, (false positive) secondary syphilis**, drug eruption, psoriasis, pityriasis rubra pilaris, lichen planus, erythema multiforme, urticaria, polymorphous light eruption, lupus erythematoses, systemic scleroderma, pemphigus seborrhoicus, Wells syndrome, rheumatoid nodule, ganglion, foreign body reaction due to ruptured infundibular cyst, folliculitis, acne, rosacea, lichen nitidus, (hypertrophic) scar, keloid, dermatofibroma, fibroma molle, connective tissue naevus, melanocytic naevi, lentigo senilis, seborrhoic keratoses, acinic keratoses, squamous and basal cell carcinomas, mycosis fungoides. **In six cases no material was left on paraffin blocks for FFM.**

### Table III.—Literature with direct detection of *Borrelia* in morphea.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Method</th>
<th>Results pos/n</th>
</tr>
</thead>
<tbody>
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<td>Aberer <em>et al.</em></td>
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<td>1987</td>
<td>Immunoperoxidase</td>
<td>7/21</td>
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<tr>
<td>Aberer <em>et al.</em></td>
<td>Austria</td>
<td>1987</td>
<td>Culture</td>
<td>1/4</td>
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<tr>
<td>Weber <em>et al.</em></td>
<td>Germany</td>
<td>1988</td>
<td>Culture</td>
<td>1/1</td>
</tr>
<tr>
<td>Aberer <em>et al.</em></td>
<td>Austria</td>
<td>1988</td>
<td>Immunoperoxidase</td>
<td>3/9</td>
</tr>
<tr>
<td>Ross <em>et al.</em></td>
<td>Puerto Rico</td>
<td>1990</td>
<td>Silver stain</td>
<td>10/25</td>
</tr>
<tr>
<td>Aberer <em>et al.</em></td>
<td>Austria</td>
<td>1991</td>
<td>Culture</td>
<td>1/11</td>
</tr>
<tr>
<td>Schempp <em>et al.</em></td>
<td>Germany</td>
<td>1993</td>
<td>PCR</td>
<td>9/9</td>
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<tr>
<td>Schempp <em>et al.</em></td>
<td>Germany</td>
<td>1993</td>
<td>PCR/immunohistochemistry</td>
<td>1/1</td>
</tr>
<tr>
<td>Weidenthaler <em>et al.</em></td>
<td>Germany</td>
<td>1994</td>
<td>PCR</td>
<td>1/1</td>
</tr>
<tr>
<td>Granter <em>et al.</em></td>
<td>USA</td>
<td>1994</td>
<td>PCR</td>
<td>1/1</td>
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<tr>
<td>Trevisan <em>et al.</em></td>
<td>Italy</td>
<td>1996</td>
<td>PCR</td>
<td>6/10</td>
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<tr>
<td>Fujiwara <em>et al.</em></td>
<td>Japan</td>
<td>1997</td>
<td>PCR</td>
<td>2/5</td>
</tr>
<tr>
<td>Breier <em>et al.</em></td>
<td>Austria</td>
<td>1999</td>
<td>Culture</td>
<td>1/1</td>
</tr>
<tr>
<td>Oxkan <em>et al.</em></td>
<td>Turkey</td>
<td>2000</td>
<td>PCR</td>
<td>3/10</td>
</tr>
<tr>
<td>Hercogova</td>
<td>Czech Republic</td>
<td>2002</td>
<td>PCR</td>
<td>1/1</td>
</tr>
<tr>
<td>Eisendle <em>et al.</em></td>
<td>Germany/Austria</td>
<td>2007</td>
<td>PCR</td>
<td>1/29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FFM</td>
<td>84/122</td>
</tr>
</tbody>
</table>

Pos. positive; N number
identified. The different studies concerning the detection of *Borrelia* in morphea have been reviewed by Weide et al. 2000, 63 and Goodland et al. 2002, 55 One major difficulty in assessing the association between morphea and borreliosis is the challenge of reliably detecting *Borrelia* in tissue specimens. These conflicting results at least in part reflect the difficulties of the various techniques used to prove/document the participation of *Borrelia* in the disease process.

Some patients with morphea show clinical similarities to classical borreliosis (Figure 6A). Early stages of morphea with a prominent lilac ring are not always easy to differentiate from EM with its active border. In late stages red-purple-brown macules of burned out morphea may mimic ACA. Remarkably, these clinical entities can occur at the same time in the same patient, and co-existent morphea has been described in patients with EM, BL, ACA and Lyme arthritis. 64-69 Moreover, there are descriptions of new patterns in borreliosis such as interstitial granulomatous dermatitis, which clinically most closely resembles morphea.70 There are also similarities in the histopathological findings (variable infiltrates of lymphocytes, macrophages and plasma cells; slight vacuolar degeneration of the basal layer in late stages; an increase of fibrocytes/fibroblasts with variable fibrosis to sclerosis) of EM and ACA on the one hand and morphea on the other.71 A bacterial etiology is further suggested because some cases of morphea respond well to antibiotic therapy, such as penicillin and ceftriaxone, D-penicillamine, discussed in older text books as inhibiting cross connections between collagen fibers, was likely to have been effective because of its metal-chelating activity which deprived the bacteria of essential trace elements such as manganese, zinc and magnesium. 76

In a recent large study of 122 morphea cases *Borrelia* could be detected in more than 68% of all cases with a significantly higher percentage (P=0.018) in active (75.0%) than inactive morphea (52.9%). This might reflect intentional or coincidental antibiotic exposure in longer existing cases and/or the natural course of disease with repression of the microorganisms by the immune system. The presence of B lymphocytes as determined by positive staining with CD20 proved to be a good diagnostic predictor for the presence of *Borrelia*, with a positive correlation of 0.85 (P<0.001). This is not surprising as the presence of B lymphocytes reflects an immune response against bacterial infections like borreliosis. The low percentage of detection of borrelian DNA with PCR in this study with one positive case in 30 (3.3%) indicates the problematic role of this technique to reliably detect *Borrelia* in tissue specimens. 77 The reason for the inconsistent results in PCR studies (positive, Table III, negative see 50, 52, 53, 55, 57, 78) could be the low number of microorganisms found in the tissue with the detection threshold being beyond for this technique. Other explanations include previous antibiotic treatment, old stage of disease, wrong biopsy site (e.g. from negative sclerotic area), or wrong fixation of tissue specimens leading to DNA cross-linking e.g. with inadequately buffered formalin. Further — except for the studies done by Ranki 1994, Dillon 1995 and Wienecke 1995 — most other negative PCR studies are lacking appropriate positive controls in terms of detection of borrelian DNA in tissue specimens from classical borreliosis such as EM, BL and ACA. 53, 78, 79 Thus, the reliability of the DNA extraction method for small DNA amounts or the PCR technique used in these studies remains somewhat debatable.

Another explanation for negative PCR results is that *B. burgdorferi* sensu latu includes *B. burgdorferi* sensu strictu, *B. garinii* and *B. afzelii* VS461, but also
newer *Borrelia* species have been identified. The pathogenic significance of these species, such as *B. valaisiana, B. hermsii, B. turicatae, B. parkeri* and most recently *B. spielmani* is not yet fully answered. While *B. burgdorferi* sensu strictu is the only well-established cause of Lyme disease in the United States, *B. afzelii, B. garinii* and probably *B. valaisiana* additionally cause “Lyme disease” in Europe and Asia. Relapsing fever borreliosis by *B. hermsii, B. turicatae and duttonii* and EM by *B. spielmani* have been described. The study by van Dam suggests that different *B. burgdorferi* genotypes have different pathogenic potentials. This is well documented for the classical borreliar manifestations, so ACA rarely occurs in the United States but is commonly seen in Europe where *B. afzelii* and *B. garinii* are more prevalent. Maybe, subspecies variations dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of morphea. Thus, another explanation for the moderate results by PCR might be that these techniques use primers highly specific for known human pathogenetic strains, while FFM uses immunhistochemistry with a less specific polyclonal antibody that probably detects most different borreliar species.

So, borreliosis is a vector transmitted disease whose causative agents, *B. burgdorferi* and variants, share collagenotropism, whereas other spirochaetes or spiral-shaped bacteria are epithelio- & endotheliotropic (*e.g.* *T. pallidum*) or mucotropic (*e.g.* *H. pylori*). Fibronectin binding proteins of *B. burgdorferi* promote bacterial attachment to glycosaminoglycans which are most prominent in connective tissue between collagen bundles. While the immune system can cope with the organisms in EM and BL comparatively quickly, in ACA and morphea the situation seems more complicated. The low level of microorganisms probably indicates that the disease is not only due to the effect of the infectious agent, but also reflects the challenge for the immune system due to the location of the microorganisms and or even a compromised immune reaction in morphea patients themselves, where *Borrelia* might trigger a subsequent autoimmune reaction. This could also explain why not all patients benefit from antibiotic therapy. Indeed, a recent study published in 2009 by Prinz JC found evidence for the induction of autoimmunity by *Borrelia* infection. The authors examined the relationship between *Borrelia* exposure, serologic autoimmune phenomena and age at disease onset in morphea patients. In 90 morphea patients the presence of *Borrelia*-specific serum antibodies was correlated to the age at disease onset and the presence and titers of antinuclear antibodies. A statistically highly significant association between morphea, serologic evidence of *Borrelia* infection, and high-titer antinuclear antibodies was observed, when disease onset was in childhood or adolescence. The conclusion was that *B. burgdorferi* infection may be relevant for the induction of a distinct autoimmune type of scleroderma, which the authors suggested to be called “*Borrelia*-associated early onset morphea”. This condition is characterized by the combination of disease onset at younger age, infection with *B. burgdorferi*, and evident autoimmune phenomena as reflected by high-titer antinuclear antibodies.

Detection of *Borrelia* in patients with lichen sclerosus

*Lichen sclerosus* (LS), frequently reported in the dermatologic literature as *lichen sclerosus et atrophicus*, is a chronic inflammatory skin disease of unknown etiology leading to substantial discomfort and morbidity. It commonly affects adult woman in the genito-anal region (Figure 7A) but also occurs elsewhere (Figure 7B). LS has clinical and histological similarities with morphea and some investigators consider this entity a superficial variant of morphea, an opinion supported by its frequent coincidence with morphea. LS further shares similarities and common features with ACA, a chronic form of borreliosis, in particular including histological findings such as an infiltrate of lymphocytes admixed with some plasma cells, an increase of fibrocytes and fibroblasts and a diffuse dermal fibrosis to sclerosis (Figures 3B, C). These observations have led several investigators to consider the possibility of *B. burgdorferi* as a common etiologic factor for both diseases. The involvement of *B. burgdorferi* as a causative agent for LS was first proposed by Aberer et al. in 1987 and subsequently further supported at least in part by several other studies (Table IV). A bacterial etiology is further suggested because several cases of LS respond well to antibiotic therapy, such as dirithromycin, penicillin and ceftriaxone. *Borrelia* have frequently been detected in Europe, but not...
Table IV.—Results of studies investigating B. burgdorferi in lichen sclerosus patients. For the PCR involving studies the amplified gene is shown in parenthesis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Method</th>
<th>Results pos./N</th>
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<td>Austria</td>
<td>1987</td>
<td>Immunoperoxidase</td>
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<td>Ross et al.48</td>
<td>Puerto Rico</td>
<td>1990</td>
<td>silver stain</td>
<td>10/21</td>
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<tr>
<td>Schempp et al.49</td>
<td>Germany</td>
<td>1993</td>
<td>PCR (Flagellin)</td>
<td>6/16</td>
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<td>Ranki et al.50</td>
<td>Finland</td>
<td>1994</td>
<td>PCR (OspA)</td>
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<td>Dillon et al.59</td>
<td>USA</td>
<td>1995</td>
<td>PCR (Flagellin)</td>
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</tr>
<tr>
<td>De Vito et al.79</td>
<td>USA</td>
<td>1996</td>
<td>PCR (Clone 2H1)</td>
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<td>Fujiwara et al.61</td>
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<td>1997</td>
<td>PCR (16s-RNA)</td>
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<td>1/10</td>
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<td>PCR (16s-RNA)</td>
<td>0/21</td>
</tr>
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<td>Colomé-Grimmer et al.62</td>
<td>USA</td>
<td>1997</td>
<td>PCR (Flagellin)</td>
<td>0/10</td>
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<tr>
<td>Alonso-Llamazares et al.63</td>
<td>Spain</td>
<td>1997</td>
<td>PCR (OspA)</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aberer et al.95</td>
<td>Austria</td>
<td>1999</td>
<td>PCR (Flagellin)</td>
<td>13/1</td>
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<td>Özkan et al.95</td>
<td>Turkey</td>
<td>2000</td>
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<tr>
<td>Breier et al.96</td>
<td>Austria</td>
<td>2001</td>
<td>Culture</td>
<td>1/1</td>
</tr>
<tr>
<td>Eisendle et al.99</td>
<td>Austria</td>
<td>2006</td>
<td>PCR (23s-RNA) FFM</td>
<td>38/60</td>
</tr>
</tbody>
</table>

Pos. positive, N number
in cases from the United States. Studies reporting a positive association between *B. burgdorferi* infection and LS found evidence of the organism in 10-68% of cases; on the other hand there are reports where no positive cases could be identified (Table IV).

In a recent large study on 61 cases of LS *Borrelia* could be detected in more than 60% of all LS cases, with a significantly higher percentage (P=0.001) in early (79.5%) than late LS (33.3%), while it made no difference if LS was associated with morphea or not (Table V). This might reflect intentional or coincidental antibiotic exposure in longer existing cases or the natural course of disease with repression of the microorganisms by the immune system. The negative detection of borrelial DNA with PCR in this study again indicates the problematic role of PCR to reliably detect *Borrelia* in tissue specimens. The low number of microorganisms beyond the detection threshold could be one explanation for the inconsistent results in PCR studies. Other explanations again include old stage of disease, wrong biopsy site (e.g. from negative fibrosclerotic parts), or wrong fixation of tissue specimens leading to DNA cross linking e.g. with inadequately buffered formalin. Further as explained above - negative PCR studies are frequently lacking appropriate positive controls in terms of detection of borrelial DNA in tissue specimens from classical borreliosis.

As in the case for morphea there is another explanation for negative PCR results as beside *B. burgdorferi* sensu latu newer *Borrelia* species have been identified, as mentioned above with unclear pathogenic significance. So, subspecies variations might dictate the clinical manifestations that follow infections, with only certain strains possessing the characteristics required to initiate the development of LS. Thus, like in the case for morphea, an other explanation for the moderate results by PCR is the specificity of the primers used working only for known human pathogenic strains, while immunohistochemistry with a less specific polyclonal antibody detects most different borrelial species.

In any case, detection of spirochetes in pure LS and LS associated with morphea seems to be a common denominator which indicates the nosologic relationship of these skin disorders.\(^{44, 61}\) Moreover, the infectious hypothesis with spirochetes helps to explain the most common stereotypical presentation of LS, namely in the genitoanal area. Subclinical dissemination with spread of *Borrelia* to kidneys and urine occurs in early borrelial infection.\(^{101, 102}\) Favoured by the moist and frequently traumatized conditions of genitalia, this might allow a superficial *Borrelia* infection in the peri-genital region, *i.e.* LS. This also explains the frequent occurrence of the disease in the perigenital area and why other mucous membranes such the oral or endonasal mucosa and conjunctiva are practically never affected. The lower level of microorganisms in late LS indicates that the disease is not only the consequence of the infectious agent, but also reflects the challenge for the immune system and or a compromised immune reaction in LS patients themselves, where *Borrelia* antigens might trigger a subsequent autoimmune reaction in genetically predisposed individuals via molecular mimicry.\(^{86}\) So, thyroid autoantibodies have been described in 36% of LS patients.\(^{103}\) *B. burgdorferi* has been proposed as environmental trigger of autoimmune thyroiditis through amino acid sequence homologies between proteins of *B. burgdorferi* and all thyroid autoantigens, like human thyrotropin receptor, human thyroglobulin, human thyroperoxidase and human sodium iodide symporter, or segments thereof.\(^{104, 105}\) As in the case of morphea the induction of autoimmunity also might explain why not all patients benefit from antibiotic therapy and make an early antibiotic treatment reasonable.

In summary, there are histopathologic similarities between classical borrelioses, morphea and LS. All this diseases show fibrosclerosis of the connective tissue and plasma cells in early inflammatory rich stages (Figures 1-3,6,7). Also clinically it is not always easy to distinguish cases of EM or ACA from early morphea (Figure 6A). Further there are clinical reports of improvement after antibiotic therapy for morphea and

<table>
<thead>
<tr>
<th>Classification of LS</th>
<th>FFM</th>
<th>PCR</th>
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<tbody>
<tr>
<td>LS (“pure”)</td>
<td>33/52 (63.5)</td>
<td>0/5 (0.0)</td>
</tr>
<tr>
<td>LS with morphea</td>
<td>5/8 (62.5)</td>
<td>0/6 (0.0)</td>
</tr>
<tr>
<td>Stage (activity) of LS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory-rich (“early”)</td>
<td>31/39 (79.5)</td>
<td>0/7 (0.0)</td>
</tr>
<tr>
<td>Inflammatory-poor (“late”)</td>
<td>7/21 (33.3)§</td>
<td>0/4 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>38/60 (63.3)</td>
<td>0/11 (0.0)</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative*</td>
<td>0/60 (0.0)</td>
<td>0/15 (0.0)</td>
</tr>
<tr>
<td>Positive**</td>
<td>61/68 (89.7)§§</td>
<td>25/68 (36.8)§§</td>
</tr>
</tbody>
</table>

FFM, focus-floating microscopy; PCR, polymerase chain reaction, LS lichen sclerosus

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**Table V.—Detection of Borrelia by FFM and PCR in LS and controls.**

Data are given as number positive/total tested (percentage).

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*Note: FFM, focus-floating microscopy; PCR, polymerase chain reaction, LS lichen sclerosus.*
LS. For these reasons and the reliable detection of spirochetal microorganisms in morphea and LS, one must conclude that at least some cases of morphea and LS should be integrated in the spectrum of cutaneous borrelioses.

**Biofilms of *B. burgdorferi sensu latu* in chronic or recurrent cutaneous borreliosis?**

The hypothesis that *B. burgdorferi* might form biofilm structures in BL and ACA was recently proposed based on the finding of large colonies of *Borrelia* in classical cutaneous borrelioses shown by immunohistochemistry and FFM. So, *Borrelia* can grow in a “medusa colony” or in a “granular colony with a reddish veil” (Figure 8 A-C). These forms of borrelial growth were first described in vitro by Abeler and Duray and such colonies reveal striking similarities to previously published biofilm pictures. It is a fascinating hypothesis to compare large borrelial aggregations in the tissues with biofilms and speculate that such biofilms of *B. burgdorferi* might be responsible for a partial resistance to antibiotic therapy in some patients with Lyme disease. Subsequently, the potential that *Borrelia* may shed from these biofilms, might thus provide a possible explanation for chronic relapsing courses of some borrelial infections. Of note, biofilm formation in the human host has already been described for other spirochetes like *Treponema denticula*, and biofilm formation has been associated with antibiotic resistance in *Helicobacter pylori* infections. Bacterial biofilms are responsible for several chronic diseases (e.g. periodontitis and chronic lung infection in cystic fibrosis patients) that are very difficult to treat because they show much greater resistance to antibiotics than their free-living counterparts. The biofilm resistance is very unique in a sense that it requires multiple mechanisms such as incomplete penetration of the antibiotics into the matrix, inactivation of antibiotics by altered chemical microenvironment within the biofilm and an altered, protected phenotypic “spore like” state of the resistant bacteria population. If *B. burgdorferi* is indeed capable forming biofilms, it will change the way, how we think about Lyme disease especially in patients,

**Figure 8.**—A) Focus-floating microscopy and immunohistochemical staining (Acris BP 1002, no counterstain, x1000) for various borrelial colony forms. Medusa-like cluster of “planktonic microorganisms” in a case of acrodermatitis chronica atrophicans. B) Colony of degenerating fragmented/small granular “dying” spirochetes in a case of morphea. C) Putative biofilm formation of a borrelial colony with a mixture of medusa-like and granular spirochetal aggregations with cystic rounded forms, tubular elements or swollen granules covered by a reddish veil in a case of lichen sclerosus.

**Figure 9.**—Summary of the various known and possible skin manifestations in cutaneous borreliosis as described in the literature. The skin diseases are subdivided for known, probable and possible borrelial etiology.

**Table:**

<table>
<thead>
<tr>
<th>Classical patterns</th>
<th>Known association</th>
<th>Partly Borrelia associated</th>
<th>Probable association</th>
<th>Single reports</th>
<th>Potential association</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Erythema (chronicum) migrans</td>
<td></td>
<td>1) Morphea/localized scleroderma</td>
<td></td>
<td>1) Cutaneous sarcoidosis</td>
<td></td>
</tr>
<tr>
<td>2) Borreli-alymphocytoma/lymphadenosis benigna cutis</td>
<td>2) Lichen sclerosus (et atrophicus)</td>
<td>2) Lichen sclerosus (et atrophicus)</td>
<td>2) Necrobiosis lipoidica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Acrodermatitis chronica atrophicans</td>
<td>3) Granuloma annulare</td>
<td>3) Granuloma annulare</td>
<td>3) Necrobiosis lipoidica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Juxta articular fibrotic nodules</td>
<td>4) Interstitial granulomatous dermatitis</td>
<td>4) Interstitial granulomatous dermatitis</td>
<td>4) Necrobiosis xanthogranuloma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
where it seems to be persistent despite antibiotics treatment.113 It would be interesting to see if patients with big borrelial colonies that might resemble biofilms are the ones that are more resistant to antibiotic treatment. More research on this topic will answer these questions.

Conclusions

The classical manifestations of cutaneous borreliosis are EM, BL — a cutaneous B-cell-pseudolymphoma (or lymphadenosis cutis benigna) — ACA and iuxtaarticular fibrinoid nodules which are related to ACA.114, 115 At least in part also other skin manifestations — as described above — especially morphea,14-217 necrobiosis lipoidica165 and cases of cutaneous B-cell lymphoma119-126 are causally related to infections with Borrelia. In the case of cutaneous B-cell lymphoma the pathogenic mechanism seems to be analogous to the one discussed for MALT lymphomas which are induced by chronic Helicobacter pylori infection.127-138 The evidence is also growing that granuloma annulare139-147 and interstitial granulomatous dermatitis148-150 might be partly caused by B. burgdorferi or similar strains. There are single reports which connect other skin diseases to Borrelia, for example cutaneous sarcoidosis, especially in the Chinese literature,151-164 then necrobiosis lipoidica,165 necrobiotic xanthogranuloma166 and cases of mycosis fungoides,167 but the evidence for the latter skin diseases is not unambiguous. In addition, as the modern chameleon of dermatology, cutaneous borrelioses, especially BL, mimic other skin conditions, as has been shown for erythema annulare centrifugum or lymphocytic infiltration of the skin (Jessner-Kanof).168-173 In summary, it can be concluded that the known spectrum of skin manifestations in cutaneous borreliosis is continuously expanding and can not be regarded as completed (Figure 7).

Riassunto

Lo spettro in espansione della borreliosi cutanea

Lo spettro conosciuto delle manifestazioni cutanee della malattia di Lyme è in continua espansione e non può essere considerato come completato. Accanto alle classiche manifestazioni della borreliosi cutanea, quali l’eritema (cronico) migrante, il linfocitoma borrelioso e l’acroderrmatite cronica atrofizzante, sta crescendo l’evidenza che almeno in parte altre manifestazioni cutanee siano correlate all’infezione da Borrelia, specialmente la morfea, il lichen sclerosus ed alcuni casi di linfoma cutaneo a cellule B. Anche il granuloma annulare e la dermatite granulomatosa interstiziale potrebbero in parte essere provocati da Borrelia burgdorferi o da ceppi simili. Vi sono anche segnalazioni isolate di altre manifestazioni cutanee associate alle infezioni da Borrelia quali la sarcoidosi cutanea, la necrobiosis lipoidica e lo xantogranuloma necrobiotico. Inoltre, come un camaleonte dermatologico, la borreliosi cutanea, specialmente nel caso del linfocitoma borrelioso, mima altre patologie cutanee, come è stato dimostrato per l’eritema annulare centrifugato o l’infiltrazione linfocitica (Jessner Kanof) della cute.


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