Interstitial granulomatous dermatitis with histiocytic pseudorosettes: A new histopathologic pattern in cutaneous borreliosis. Detection of *Borrelia burgdorferi* DNA sequences by a highly sensitive PCR-ELISA

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Background: The cutaneous manifestations of *Borrelia burgdorferi* infection include an early phase of erythema chronicum migrans and a late stage of acrodermatitis chronica atrophicans lesions.

Objective: We describe 11 patients with peculiar cutaneous manifestations and distinctive histopathologic findings as the result of *B burgdorferi* infection.

Methods: Eleven patients with *B burgdorferi* detected by polymerase chain reaction or polymerase chain reaction enzyme-linked immunosorbent assay in their cutaneous lesions were included in this study. We analyzed clinical data and histopathologic findings in all patients. The inflammatory infiltrate was also immunohistochemically investigated.

Results: Most patients showed a peculiar clinical setting of morphea, and a few cases presented the characteristic appearance of erythema chronicum migrans instead of acrodermatitis chronica atrophicans, as would be expected in a late phase of *B burgdorferi* infection. The histopathologic findings were similar in all cases and consisted of an interstitial inflammatory infiltrate mostly composed of histiocytes dispersed among the collagen bundles of the dermis and focal areas of small pseudorosette formation, characterized by small histiocytes radially disposed around thick collagen bundles. In some cases there were also a few plasma cells intermingled with the histiocytes.

Conclusion: Cutaneous lesions with clinical appearance similar to that of morphea and histopathologic features closely resembling those of the interstitial type of granuloma annular may be seen in intermediate-stage cutaneous lesions of *B burgdorferi* infection. These clinical and histopathologic findings represent a constellation of findings that have not been previously characterized as a cutaneous manifestation of *B burgdorferi* infection. (J Am Acad Dermatol 2003;48:376-84.)

In 1982 Burgdorfer et al[1] isolated a spirochete, now called *Borrelia burgdorferi*, as the microorganism implicated in Lyme disease. Shortly after, it was demonstrated and accepted worldwide as the agent responsible for all cutaneous manifestations of this disease. Lyme disease was first described in 1977 by Steere et al[2] as a multisystemic disorder that affects primarily the skin, nervous system, heart, and joints. The most common and most specific manifestations of Lyme disease are dermatologic lesions,[3-7] including an early phase of erythema chronicum migrans (ECM) and a late stage of acrodermatitis chronica atrophicans (ACA) lesions. It is important to recognize the cutaneous manifestations of Lyme disease as soon as possible, because ECM usually responds well to oral antibiotic therapy; if untreated, the infection may continue for many years and develop the irreversible degenerative changes of ACA. In the early infection,[8-10] the characteristic lesions of ECM usually start at the site of a tick bite as an erythematous macule or papule that

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spreads centrifugally, resulting in an annular erythematous lesion with central clearing. After weeks to months, secondary lesions of ECM may appear far from the tick bite site as an expression of hematogenous dissemination of the spirochetes. Secondary lesions of ECM are multiple in approximately 25% of the cases and often are nonexpanding, smaller, and more homogeneous than the primary lesions of ECM. After months to years, lesions of ACA may develop, usually starting on the same part of the body initially involved by the ECM; therefore, the extremities are the most common locations. Clinically, the lesions of ACA begin as a diffuse or localized bluish red edematous erythema, which gradually spreads favoring the extensor surfaces of the extremities and the areas around the joints where fibrotic nodules may appear.[11-15] Finally, these changes are replaced by a gradual and marked atrophy of the skin with loss of appendages and often accompanied by hypopigmentation.

In addition to these characteristic cutaneous manifestations of Lyme disease, *B burgdorferi* has been implicated by some authors in a large series of disparate cutaneous disorders such as morphea,[16-22] lichen sclerosus et atrophicus,[18] septal panniculitis resembling erythema nodosum,[23] porphyria cutanea tarda with sclerodermic changes,[24] pseudolymphomas,[25] eosinophilic fasciitis (Shulman syndrome),[26-28] and progressive facial hemiatrophy of Parry-Romberg.[29] Nevertheless, a review of the literature reveals that not all authors agree on the relationship of *B burgdorferi* with these latter disorders,[30,31] and up to now the exact implication of *B burgdorferi* in these entities remains uncertain.

In this article, we describe the cutaneous manifestation of a possible intermediate stage between ECM and ACA in *B burgdorferi* infection, with peculiar clinical appearance and distinctive histopathologic findings. Eleven patients were included in this study, with *B burgdorferi* detected by polymerase chain reaction (PCR) or PCR enzyme-linked immunosorbent assay (ELISA). Most of them showed a peculiar clinical setting of morphea, and a few cases presented the characteristic appearance of ECM instead of ACA, as would be expected in the late phase. Despite these clinical manifestations, in all cases the histopathologic findings were similar to those seen in the interstitial type of granuloma annulare: a predominant but variable account of histiocytes dispersed among the collagen bundles of the dermis and focal areas of small pseudorosette formation, characterized by small histiocytes radially disposed around thick collagen bundles. These clinical and histopathologic findings represent a constellation of findings that have not been previously characterized as a cutaneous manifestation of *B burgdorferi* infection.

MATERIAL AND METHODS

Thirteen cutaneous biopsy specimens from 11 patients were fixed in 10% buffered formalin. In all the cases, paraffin-embedded sections were stained with hematoxylin and eosin, periodic acid-Schiff, and orcein. Immunohistochemical studies of the inflammatory infiltrate were performed using the following antibodies: CD68 for histiocytes (PGM1, Dako, Glostrup, Denmark), CD3 for T cells (CD3 epsilon, polyclonal, Dako), CD20 for B cells (L26, Dako), CD79a for B cells and plasma cells (CD79a, Dako), VS 38c for plasma cells (VS 38c, Dako), and neutrophil-elastase for neutrophils (Dako).

Serology tests (IgM and IgG-ELISA) for *B. burgdorferi* were performed in all patients. In case 1, serology was repeated 2 times after the patient received antibiotic treatment.

For molecular identification of *B burgdorferi,* DNA was prepared from paraffin-embedded tissue. In each case, 10 sections, each 10-μm thick, were studied. Deparaffinization was performed for 15 minutes per step, shaking at 55°C with xylene and 1 minute washing with 100% ethanol (altogether, approximately 45 minutes), and digestion with 0.6 mg Proteinase K for 16 hours. The remaining DNA was purified by adsorption chromatography (QIAamp DNA Mini Kit, QIAGEN GmbH, Hilden, Germany), and the concentration of the sample was adjusted to 10 ng/μL. Nested PCR was performed in volumes of 25 μL with 50 ng DNA, 100 pmol of each primer, 10 mM TRIS-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 200 mM of each dNTP, and 1.5 units *Taq*-Polymerase (native *Taq*-Polymerase from *Thermus aquaticus*). The samples were subjected to the following conditions: for the first PCR: 30 seconds at 94°C, 30 seconds at 53°C, and 30 seconds at 72°C for 40 cycles; for the second PCR: 30 seconds at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C for 45 cycles in a PTC 200 thermocycler (MJ Research, Inc, Watertown, Mass). For amplification, the following primers specific for the *B burgdorferi* 23 S rRNA gene[32] were used: for the first PCR: Bor-1: 5′-AGAAGTGCT GGAGTCGA-3′, Bor-2: 5′-TAGTGCTCTACCTCTAT TAA-3′; for the second PCR: Bor-3: 5′-GCGAAAGC GAGTCTTAAAAGG-3′, Bor-4: 5′-ACTAAAATAAG GCTGAACTTAAAT-3′. After separation on a 2% horizontal agarose gel (50 mA for 30 minutes) (agarose for electrophoresis: NEEO from Roth, Karlsruhe, Germany molecular biology grade) and staining with ethidium bromide, the PCR product of 219 base pairs was visualized under ultraviolet light (302 nm). Although the amount of DNA after the first step of

TABLE I. Clinical features of patients with *Borrelia burgdorferi*-induced cutaneous lesions and histopathologic findings similar to those of interstitial granuloma annulare

Patient No.	Age (y)/Sex	Location/clinical appearance	Clinical diagnosis
1	75/F	Breast, abdomen, leg and groin: progressive erythematous palm-sized lesions	Morphea Borreliosis
		For 1 y (doxycyclin 200 mg/day for 2 wk without effect)	Granuloma annulare Drug eruption
		2nd biopsy: 9 mo later	Same as above
		3rd biopsy: 22 mo later	Same as above
2	2/F	Interscapular: annular erythema	Erythema chronicum migrans
3	62/M	Trunk and extremities: multiple annular erythema	Multifocal erythema chronicum migrans Figurate erythema
4	78/F	Right thigh (extensor): solitary erythematous plaque	Borreliosis
5	43/F	Abdomen: solitary erythematous lesion with elevated borders	Microbial eczema Tinea profunda
		No pruritus	Granuloma annulare
6	56/F	Both groins and adjacent extensor areas of the thighs: livid progressive macules and erythema, for 6 mo	Lichen sclerosus et atrophicus No pruritus Morphea
		No pruritus "Rheumatic disease" (ANA+)	Dermatomyositis
7	49/F	Abdomen: solitary indurated erythema	Parapsoriasis Cutaneous lymphoma
8	60/F	L side of the thorax (submammary): indurated erythema	Morphea
9	59/F	Abdomen: two adjacent lesions of annular erythema with elevated borders	Erythema annulare centrifugum Lichen sclerosus et atrophicus Morphea Cutaneous lymphoma
10	54/F	L thigh and groin, both axillae: progressive figurate erythema	Erythema annulare Morphea
11	64/F	Both legs: diffuse, slightly indurated/urticarial erythema, for 4 y	Morphea

L, Left.

PCR could only be estimated (because the amount is not visible on an agarose gel and cannot be spectroscopically measured), it should be less than 10 ng of the PCR product (approximately the amount visible on an agarose gel). We used 2 μL (approximately one tenth) of the first reaction for the second PCR.

Additional PCR-ELISA was performed only in those cases with previous PCR negative results. For the PCR-ELISA, 2.5 μL of DIG-labeling mix (Roche Diagnostics, Mannheim, Germany) was added to the second PCR reaction mix, and the volume was adjusted to 25 μL. After evaluation of the samples on a 2% agarose gel, 10 μL of the PCR reaction was denatured for 10 minutes, and the PCR products were hybridized to a biotin-labeled probe (concentration 25 ng/μL) for 3 hours in a streptavidin-coated microtiter plate. Binding of the antibody and color detection was performed following the instructions of the supplier (Roche Diagnostics).

RESULTS

The clinical data of the 11 patients are shown in Table I. Briefly, there were 10 female patients and 1 male patient, ranging in age from 2 to 78 years (mean age, 54 years). Six patients showed multiple erythematous cutaneous lesions located on the trunk or extremities or both, most of them with a clinical appearance of large patches similar to those of the inflammatory stage of morphea (Fig 1). The lesions had several months of evolution, and they were either stationary or slowly progressing, just like morphea. The other 5 patients presented a solitary, usually indurated, plaque-like lesion, with the trunk as the most common location.

The histopathologic and molecular biologic findings are summarized in Table II. All biopsy specimens showed essentially the same findings, which consisted of a superficial and deep perivascular and interstitial dermal inflammatory infiltrate with epi-

dermal and subcutaneous fat sparing (Fig 2, *A*). Only 1 patient (case 5) presented epidermal involvement with exocytosis of lymphocytes accompanied by an underlying superficial lichenoid dermatitis. The infiltrate ranged from sparse to abundant, conferring to the dermis a "busy" appearance at lower power, even in the cases with fewer inflammatory cells. This infiltrate was mostly composed of interstitially arranged small histiocytes (Fig 2, *B*) and perivascularly disposed lymphocytes. In some patients (cases 5, 10, and 11), there were also scattered eosinophils or plasma cells. The interstitial histiocytes were located in the mid and deep dermis and interspersed among the collagen bundles with associated slight deposits of mucin. There was no evidence of enlarged histiocytes, fibrosis, or significant changes of the collagen bundles either as homogeneous thickening or as basophilic degeneration. The elastic fibers were either conserved or slightly reduced. Only 1 patient (case 9) exhibited a few superficial foci of necrobiotic granulomas centered by copious amounts of mucin and degenerated bundles of collagen with a surrounding rim of histiocytes arranged in palisade. In addition to these features, 4 patients (cases 4, 5, 8, and 11) showed a striking finding mainly seen in the deeper reticular dermis. It was characterized by the presence of small granulomatous pseudorosettes composed of a "free-floating" collagen bundle entirely surrounded by histiocytes, which sometimes displayed a palisade array (Fig 2, *C*).

Immunohistochemical studies basically demonstrated the same results in all cases. They consisted of CD68 positivity in the histiocytes (Fig 3), whereas most of the lymphocytes expressed CD3 immunoreactivity along with a minority of CD20-, CD79a-, or VS 38c-positive cells.

In regard to the molecular biological findings, *Borrelia* serology (IgM and IgG-ELISA) was negative or not conclusive in all cases. Only 6 patients (cases 2, 3, 4, 5, 10, and the first biopsy of case 1) were positive with standard PCR method. We then performed additional PCR-ELISA investigations, which are 50- to 100-fold more sensitive than conventional PCR, in the remaining 5 negative biopsy specimens (cases that were directly positive with the first PCR had no need to have additional PCR-ELISAs) (Fig 4). This method yielded positive results in the 5 patients (cases 6, 7, 8, 9, and 11). Case 1 was quite remarkable showing positivity with PCR in the first biopsy and becoming negative with both PCR and PCR-ELISA in the remaining 2 biopsy specimens taken after antibiotic treatment. Nevertheless, the 3 biopsy specimens showed the previously described interstitial and diffuse pattern of inflammatory infiltrate and the clinical appearance of morphea.

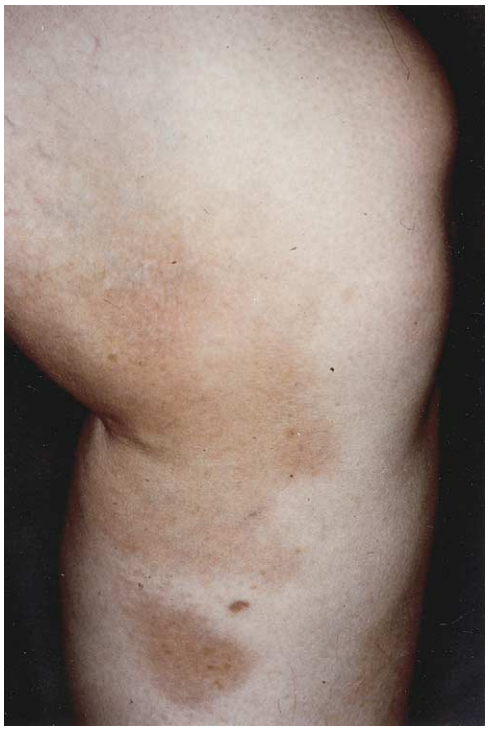


Fig 1. Case 1. Clinical appearance of the lesions consisted of areas of hyperpigmentation resembling early morphea.

DISCUSSION

We report a series of 11 patients with cutaneous lesions resulting from *B burgdorferi*, demonstrated either by PCR or PCR-ELISA, who presented a peculiar clinicopathologic constellation of findings. Clinically, many of the cases showed large erythematous patches, mostly located on the trunk, in a pattern that resembled that of the lesions of morphea in its inflammatory stage. Nevertheless, all cases revealed essentially the same histopathologic features, which consisted of a predominantly interstitial inflammatory infiltrate mainly composed of histiocytes interspersed among the collagen bundles in concert with small deposits of mucin. These histopathologic findings closely resembled those of the interstitial type of granuloma annulare. Moreover, one of the cases showed a combined pattern of classic granuloma annulare, with the necrobiotic granuloma located in the upper part of the dermis and the interstitial pattern involving the mid and reticular dermis. In addition to these characteristic findings, some cases also showed some unusual peculiarities, including the presence of granulomatous pseudorosettes in the deeper dermis and, in other cases, the presence of a few plasma cells. Although the latter was not a conspicuous finding, it is quite unusual in the conventional granuloma annulare and probably was related to the presence of *B burgdorferi* in the le-

Table II. Histopathologic and microbiologic findings in patients with cutaneous lesions induced by *Borrelia burgdorferi*

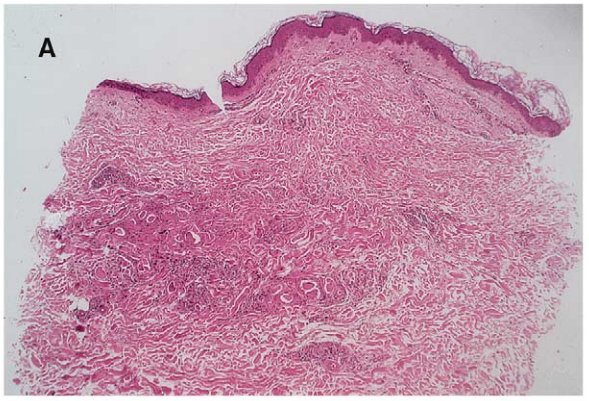
Case No.	Histopathologic diagnosis	Additional histopathologic findings	PCR	PCR-ELISA
1	GA-like (interstitial type) ++: Histiocytes		+	ND
	GA-like (interstitial type) ++: Histiocytes	Sparse interstitial mucin	–	–
	GA-like (interstitial type) ++: Histiocytes	Sparse interstitial mucin	–	–
2	GA-like (interstitial type) +: Histiocytes		+	ND
3	GA-like (interstitial type) ++/+++: Histiocytes	Sparse interstitial mucin Slight decrease in elastic tissue	+	ND
4	GA-like (interstitial type) ++/+++: Histiocytes Granulomatous rosettes	Sparse interstitial mucin Slight decrease in elastic tissue	+	ND
5	GA-like (interstitial type) ++/+++: Histiocytes +: Eosinophils Granulomatous rosettes Superficial lichenoid dermatitis with exocytosis of lymphocytes	Sparse interstitial mucin	+	ND
6	GA-like (interstitial type) +/++: Histiocytes	Slight decrease in elastic tissue	–	+
7	GA-like (interstitial type) +/++: Histiocytes	Sparse interstitial mucin	–	+
8	GA-like (interstitial type) ++: Histiocytes Granulomatous rosettes	Sparse interstitial mucin Slight decrease in elastic tissue	–	+
9	GA-like (interstitial and necrobiotic types) ++/+++: Histiocytes	Sparse interstitial mucin Slight decrease in elastic tissue	–	+
10	GA-like (interstitial type) ++: Histiocytes +: Plasma cells	Sparse interstitial mucin	+	ND
11	Granuloma annulare-like (interstitial type) ++: Histiocytes +: Plasma cells Granulomatous rosettes Deep morphea-like sclerosis		–	+

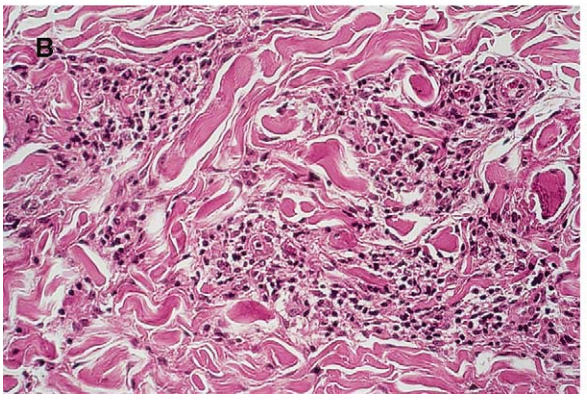
GA, Granuloma annulare; *ND*, not done; *PCR-ELISA*, polymerase chain reaction–enzyme-linked immunosorbent assay.
Inflammatory cells: +: sparse number; ++: moderate number; +++: abundant number.

sions. Therefore it might be a histopathologic clue for the right diagnosis.

The cause of morphea and granuloma annulare remains unknown and controversial, although *B burgdorferi* has been involved in both, particularly in patients who live in endemic areas. In fact, in the older literature there are reports of granuloma annulare that responded to penicillin. Moreover, examples of the coexistence of both types of lesions in the same patient have been recently reported, reflecting a possible common causative link, although *B burgdorferi* was not detected.[33,34] Nevertheless, to our knowledge, the occurrence of combined findings of granuloma annulare and morphea has not been previously described in a lesion in which *B. burgdorferi* has been demonstrated.

Granuloma annulare is a distinctive dermatosis that affects female subjects more often and occurs at





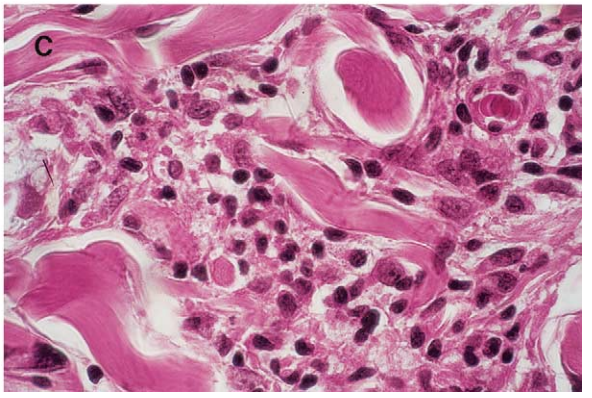
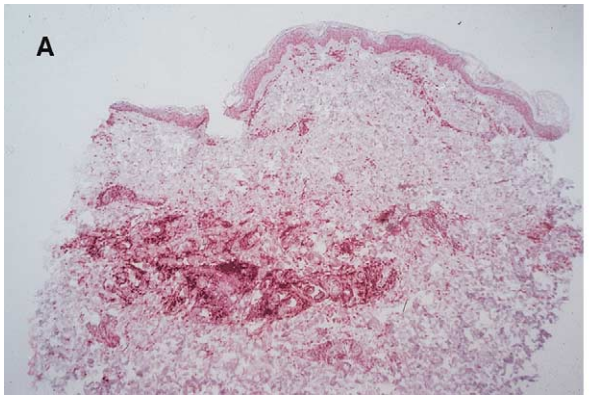


Fig 2. Case 8. Histopathologic features. **A,** Superficial and deep perivascular and interstitial dermal inflammatory infiltrate. **B,** This infiltrate was mostly composed of interstitially arranged small histiocytes. **C,** Granulomatous pseudorosettes composed of a "free-floating" collagen bundle, entirely surrounded by histiocytes. (**A-C,** Hematoxylin-eosin stain; original magnifications: **A,** ×20; **B,** ×100; **C,** ×400.)



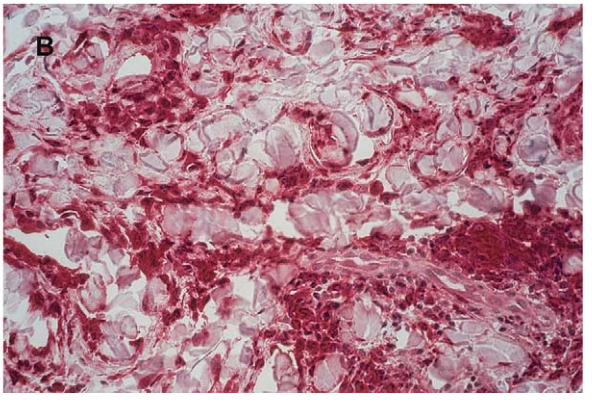


Fig 3. Case 8. **A,** Immunohistochemical studies demonstrated that most cells of the inflammatory infiltrate were CD68-positive histiocytes. **B,** Higher magnification showing the cytoplasmic CD68 immunoreactivity of the interstitially arranged histiocytes. (**A** and **B,** Immunohistochemical stain for CD68; original magnifications: **A,** ×20; **B,** ×100.)

any age. There are different clinical variants, but the localized one is the most common presentation. It consists of grouped, sometimes erythematous, smooth papules that involve more often the extensor aspects of extremities and have a tendency to form annular plaques. Histopathologically, the conventional form of granuloma annulare may exhibit basically two patterns, the necrobiotic and the inter-

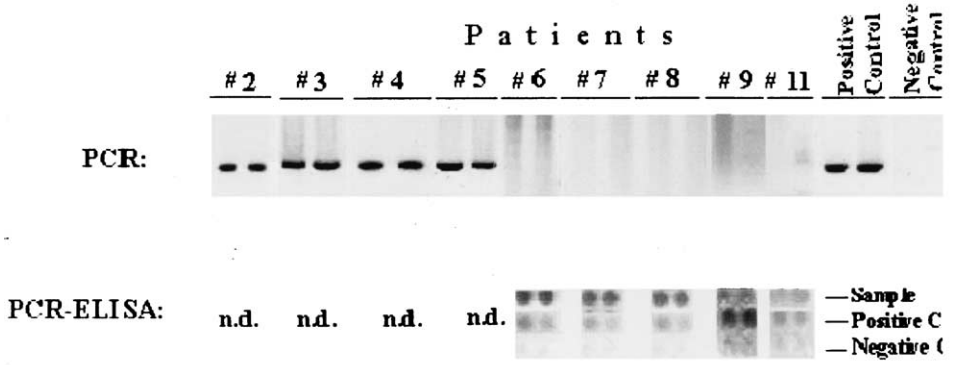


Fig 4. Results of the *B burgdorferi* polymerase chain reaction (PCR)/PCR enzyme-linked immunosorbent assay (ELISA) investigations. PCR products of samples 2, 3, 4, 5, 6, 7, 8, 9, and 11 were loaded on a 2% agarose gel (top). Additional PCR-ELISA of samples 6, 7, 8, 9, and 11 (bottom). The dark round wells on the microtiter plates indicate positive results of the PCR-ELISA. *n.d.,* Not done.

stitial types, which may appear separately or combined in the same lesion. The interstitial type is at least as common as the necrobiotic type. The lesions consist of histiocytes interstitially arranged between the collagen bundles of the dermis in the former, and foci of histiocytes arranged in palisade around degenerated bundles of collagen in the latter. Deposits of mucin are often seen in both patterns. These granulomatous foci are usually accompanied by discrete clusters of superficial and deep perivascular lymphocytes and sometimes by scattered eosinophils. Plasma cells, as already mentioned, are rare. One of the features that distinguishes the necrobiotic type of granuloma annulare from other necrobiotic granulomas (ie, necrobiosis lipoidica) is the presence of preserved areas of the dermis between the granulomatous foci.[35]

Our cases consisted histopathologically of an interstitial inflammatory infiltrate of histiocytes and, in addition to the interstitial type of granuloma annulare, the differential diagnosis included other histopathologically similar dermatoses such as interstitial granulomatous dermatitis with arthritis (IGDA) and the methotrexate-induced rheumatoid papules. IGDA is a rare condition, first described in 1965 by Dykman et al[36] as an unusual rheumatoid granuloma in the form of linear subcutaneous bands that appeared in patients with long-standing severe rheumatoid arthritis. Some authors consider IGDA to be a distinctive disorder associated with rheumatoid arthritis,[37] whereas other investigators believe that it is just one of many manifestations of the wide clinicopathologic spectrum of cutaneous disorders associated with collagen vascular diseases.[38] Clinically, the cutaneous lesions of IGDA appear as slightly red or skin-colored cords in a linear arrangement ("rope-sign") usually located on the lateral trunk. Histopathologically, these lesions share with the interstitial type of granuloma annulare the presence of a diffuse and predominantly histiocytic infiltrate that in some small foci is arranged in palisades around degenerated basophilic collagen. In contrast with the interstitial type of granuloma annulare, however, the lesions of IGDA show a denser, bottom-heavy inflammatory infiltrate of histiocytes intermingled with some neutrophils and eosinophils. Moreover, many of the histiocytes have large, pleomorphic nuclei, and mitotic figures are not uncommon.[39]

Methotrexate-induced rheumatoid papules have been recently described by Goerttler et al[40] as a distinctive cutaneous adverse reaction that appears shortly after administration of relatively high doses of methotrexate in patients with collagen vascular diseases. Clinically, the lesions consisted of erythematous grouped papules and patches with an insect bite-like appearance, mostly located on the proximal areas of the extremities. The histopathologic findings revealed a diffuse interstitial inflammatory infiltrate, mainly composed of histiocytes and a few neutrophils that (in contrast with the interstitial type of granuloma annulare) involved the full thickness of the dermis without a top-heavy pattern and presented small granulomatous foci of histiocytes in a palisaded array around central clusters of neutrophils instead of degenerated basophilic collagen. Interestingly, in the deeper areas of the reticular dermis of some of the cases, small granulomatous pseudorosettes consisting of tiny aggregations of histiocytes also arranged in palisade but around a thick collagen bundle could be detected. Our patients showed no history of methotrexate intake.

From a clinical point of view and taking into

account the presence of *B burgdorferi* in the lesions of our patients, morphea, ECM, and ACA should also be considered in the differential diagnosis. The early or inflammatory stage of morphea is clinically characterized by one or several erythematous indurated plaques with a lilac hue on the trunk or the extremities. In the late or sclerotic stage, lesions become white or yellow and show loss of cutaneous appendages and pigmentary changes. Histopathologic studies of the lesions in the inflammatory stage reveal subtle or no changes in the collagen bundles and a predominantly perivascular inflammatory infiltrate composed of lymphocytes, plasma cells, eosinophils, and sometimes neutrophils. This infiltrate is denser in the lower half of the reticular dermis and usually extends to the subcutaneous fat as a septal panniculitis. In the sclerotic stage, the most striking feature consists of the thickening of the dermis with broad sclerotic eosinophilic collagen bundles (nearly without clefts) and oriented in parallel or following thickened subcutaneous septa. Elastic stains demonstrate that elastic fibers are spared.[41]

ECM and ACA have a clinical appearance quite different from that of the patients described in this article. Histopathologically, the lesions of ECM show a rather nonspecific inflammatory pattern, characterized by a slight to moderate, mostly superficial and deep perivascular inflammatory infiltrate of lymphocytes with variable amounts of plasma cells and eosinophils.[42,43] On the other hand, the lesions of ACA[14,44] reveal, in an initial stage, a dermal lymphocytic cell infiltrate with a rich admixture of plasma cells in a band-like fashion or accentuated around telangiectatic blood vessels and adnexa. In late-stage lesions, there is intense dermal atrophy accompanied by loss of elastic fibers and folliculosebaceous units, with a variable epidermal atrophy, which may show basal vacuolar change and hyperkeratosis.

In summary, we describe 11 patients with peculiar cutaneous lesions in which PCR or PCR-ELISA methods demonstrated the presence of *B burgdorferi*. Although they were not early ones, the lesions had not yet progressed to ACA. Furthermore, they showed a striking, peculiar clinicopathologic combination of features; the clinical picture was similar to that of the inflammatory stage of morphea. The histopathologic findings were those of the diffuse type of granuloma annulare, with two peculiarities, namely, the presence in some cases of a few plasma cells or granulomatous pseudorosettes or both. We believe that in *B. burgdorferi* endemic areas, this combination of clinical and histopathologic features should be recognized as one of the many manifestations of *B burgdorferi* infection. It must be added, however, that identical histopathologic features may be seen in lesions with negative results for *B burgdorferi* by conventional PCR investigations. Obviously, they might represent the diffuse type of granuloma annulare, but in long-standing infections of borreliosis the number of microorganisms may be exceedingly scant (as they are in lesions of ACA in which standard PCR is negative in most of the cases). Thus, when there is a strong suspicion of borreliosis, the cutaneous biopsy should be taken from the outer margins of the lesion, which is the place where most of the microorganisms may be encountered, and a highly sensitive PCR-ELISA should be also performed.

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