

Anetoderma: Another facet of Lyme disease?

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Lyme disease has been suspected to be one cause of secondary anetoderma. We present a 25-year-old male patient with multiple lesions of anetoderma with a diameter of up to 2 cm that developed within the last 4 weeks without subjective symptoms. The histopathologic pattern was similar to the inflammatory stage of acrodermatitis chronica atrophicans. Polymerase chain reaction analysis out of the paraffin-embedded tissue, confirmed by sequencing of the obtained nucleotide product, revealed a part of the 23 S ribosomal RNA gene of Borrelia burgdorferi sensu lato. Enzyme-linked immunosorbent assay showed an increased serum IgG titer against B burgdorferi corroborated by Western blot analysis. After a treatment with oral doxycycline (100 mg twice a day) for 30 days the anti-B burgdorferi titer decreased significantly and no new lesions appeared. Some cases of anetoderma might be caused by Borrelia and patients with anetoderma should be examined for borreliosis including serology and polymerase chain reaction of lesional skin. In cases of Borrelia-induced anetoderma, early antibiotic treatment could prevent further progression of the disfiguring skin lesions and the underlying disease. (J Am Acad Dermatol 2003;48:S86-8.)

Anetoderma is a rare skin disease with a circumscribed loss of elastic tissue resulting in a palpable loss of dermal substance. Primary forms without underlying disease and secondary forms as a result of associated conditions have been described. Anetoderma seems to be more frequent in central Europe, suggesting a possible relationship to chronic atrophic acrodermatitis.¹ However, to our knowledge, *Borrelia* has not been identified in lesional skin by culture or molecular biologic techniques.

CASE REPORT

A 25-year-old male patient presented himself with a history of multiple circumscribed skin lesions that had developed within the last 4 weeks, without causing any subjective problems. He did not notice any tick bite or migratory erythema. On examination, multiple, grouped, round to elliptic, well-defined, atrophic macules with a diameter of 1 to 2 cm were found, distributed symmetrically on the extensor surface of the elbows, shoulders, and neck. The skin surface appeared thinner and wrinkled, with discrete telangiectases (Fig 1). No other skin changes or symptoms were found in the patient, who appeared healthy. On the basis of the skin lesions, the diagnosis of anetoderma was made.

Histopathology of an early lesion showed a patchy lymphoplasmocytic infiltration throughout the dermis with small aggregates of histiocytes (Fig 2). With an orcein-elastic stain, a focal loss of elastic fibers could be demonstrated (Fig 3). This histopathologic pattern is seen in the inflammatory stage of acro-

dermatitis chronica atrophicans.² Therefore, polymerase chain reaction (PCR) studies for the detection of DNA of *B burgdorferi* in sensu lato were done. (Histopathology and PCR studies were done in the Dermatopathology practice in Friedrichshafen.)

For molecular identification of *B burgdorferi* in sensu lato DNA was prepared from paraffin-embedded tissue. After deparaffinisation with xylol and ethanol, 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/ μ L. Nested PCR was performed in volumes of 25 μ L with 50 ng DNA; 100 pmol of each primer; 10 mmol/L TRIS-HCl, pH 9.0; 50 mmol/L KCl; 1.5 mmol/L MgCl₂; 200 mmol/L of each dNTP; and 1.5 U Taq-polymerase. The samples were then 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 45 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 thermocycler (PTC 200, MJ Research Inc, Watertown, Mass). For amplification the following primers specific for the highly conserved *B burgdorferi* in sensu lato 23 S ribosomal RNA gene³ were used: (for the first PCR) Bor-1, 5'-AGAAGTGCTG-GAGTCGA-3', and Bor-2, 5'-TAGTGCTCTACCTCTATTAA-3'; and (for the second PCR) Bor-3, 5'-GCGAAAGCGAGTCTTAAAGG-3', and Bor-4, 5'-ACTAAAATAAGGCTGAACCTTAAAT-3'. After separation on a 2% agarose gel (50 mA for 30 minutes) and staining with ethidium bromide, the PCR product of 219 base pairs was visualized under UV light (302 nm). DNA from paraffin-embedded tissue of a patient with a *B burgdorferi* infection was used as a positive control and both a sample free of DNA and a sample free of *B burgdorferi* DNA were used as negative controls. To exclude the possibility of amplification of an unspecific DNA sequence leading to a PCR product of the expected length, the amplification product was first extracted from the gel (QIAquick PCR purification kit, Qiagen GmbH) following the instructions of the suppliers and then sequenced by the dideoxytermination method (GATC, Konstanz, Germany).⁴ Thereby, our previous results could be confirmed as a comparison of the obtained nucleotide sequence to gene bank sequences⁵ identifying the PCR product as part of the 23 S RNA gene of *B burgdorferi*. Since *B burgdorferi* in sensu lato is



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Fig 1. Typical anetoderma lesion on extensor side of left elbow.

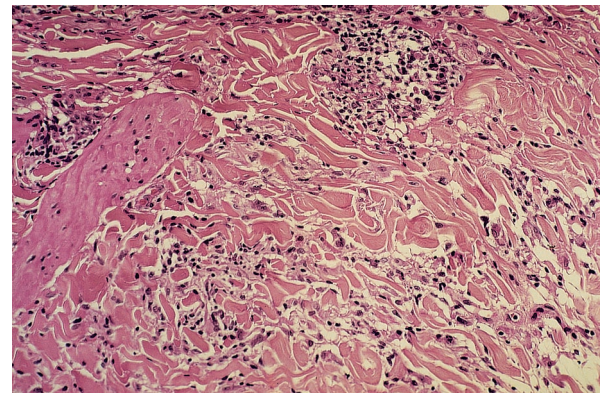


Fig 2. Patchy lymphoplasmocytic infiltrate throughout dermis with small aggregates of histiocytes. (Hematoxylin-eosin stain; original magnification $\times 100$.)

divided into different subtypes (the species *B burgdorferi* in sensu stricto, *B afzelii*, and *B garinii*),^{6,7} we tried to identify the subtype by PCR amplifying the highly variable internal transcribed spacer region. Therefore, a seminested PCR with one of the primers Borits-1A, 5'-AAGTT(A/G)TTGCCAGGGTTTT(A/G)-3', Borits-1B, 5'-AAGTT(A/G)TTGCCAGGGTTTT-3', Borits-1C, 5'-TTGCCAGGGTTTT(A/G)TTTTAT-3', or Borits-1D, 5'-CTGCGAGTTCGCGGGAGAGT-3' as forward primers and Borits-2, 5'-TAGACTCTTACTTTGAC-3' as reverse primer for the outer PCR and the same forward primers and Borits-3, 5'-TTACTTTGACCATATTTTA-3' for the inner PCR was performed with a denaturation temperature of 94°C for 30 seconds, annealing temperatures of 48°C, 50°C, 52°C, and 56°C for 30 seconds; and 72°C extension for 30 seconds for 45 cycles each. The products of both outer and inner PCR were sequenced with dye terminators (ThermoSequenase dye terminator kit, Amersham Pharmacia Biotech, Uppsala, Sweden) on an automated sequencer (AlfExpress, Amersham Pharmacia Biotech) following the instructions of the suppliers. Sequencing of the amplified fragment showed a pattern, which seemed to be derived from a mixture of amplification products of the same length.

At time of first presentation, laboratory evaluation using enzyme-linked immunosorbent assay showed an increased IgG titer (88 U) against *B burgdorferi*, normal IgM values (9 U), and an increased blood sedimentation rate (51/85). This results were confirmed by Western blot showing IgG antibodies against p83, flagellin, outer surface protein C of *B burgdorferi* OspC, and OspD, and IgM antibodies against flagellin and OspA. Treatment with oral doxycycline (100 mg twice a day) for 30 days was started and no new lesions appeared. Existing lesions remained unchanged, however. The blood sedimentation rate declined to a normal value. The *Borrelia* IgG titer had risen to 108 U when the treatment was finished, and it had decreased to 30 U by the end of the following 2 months. The patient did not give his consent for serologic HIV testing; however, we found no clinical signs for HIV infection.

DISCUSSION

Lyme disease is a multisystem disease involving mainly the skin, heart, joints, and nervous system. Diagnosis is based on clinical manifestations; the most specific symptoms are dermatologic ones such as migratory erythema, lymphadenosis benigna cutis, and chronic acrodermatitis. To prevent late sequelae affecting the joints, heart, and nervous system, early diagnosis of borreliosis is important.⁸ Anetoderma is a rare dermatologic

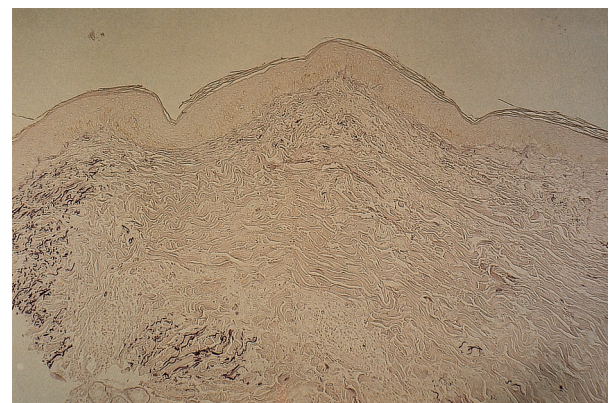


Fig 3. Focal loss of stainable elastic fibers in dermis. (Orcein stain; original magnification $\times 40$.)

disease manifesting with a localized laxity of the skin as a result of a loss of dermal elastic fibers. It has been observed that the underlying tissue damage is related to autoimmune mechanisms (eg, systemic lupus erythematosus, chronic discoid lupus erythematosus, and antiphospholipid syndrome), to infections (eg, syphilis, tuberculosis, and HIV), or to other diseases such as urticaria pigmentosa, lymphocytoma cutis, and nodular amyloidosis. A relationship between anetoderma and *Borrelia* infection has been suspected; however, investigations yielded contradictory results.¹ Furthermore, treatment of anetoderma with penicillin has been of variable success.^{9,10} In a single case, treatment with ceftriaxone sodium stopped progression of the disease suggesting an infectious origin.¹¹ The investigation of the role *B burgdorferi* might play in anetoderma is confounded by false-negative and false-positive serology tests.¹² In our case, both histopathology and serology implicates an causative role of *B burgdorferi* in sensu lato, supported by the detection of *B burgdorferi*-DNA in sensu lato in lesional skin and by successful antibiotic treatment. However, subtyping of *B burgdorferi* in sensu lato by sequencing of the PCR product of the highly variable internal transcribed spacer region to identify either *B burgdorferi* in sensu stricto, *B afzelii*, or *B garinii* showed a mixture of fragments. This can be explained by an infection with different strains of the subspecies of *B burgdorferi* in sensu lato,^{13,14} whereas the clear sequence of the 23S ribosomal RNA gene implicates to be the result of the amplification of a region that is highly conserved in all strains.

B burgdorferi in sensu stricto prevails in *Borrelia* infection acquired in the United States. Acrodermatitis chronica atrophicans is rare in the United States, whereas it is common in Europe, where different strains of *Borrelia* (*B burgdorferi* in sensu stricto, *B afzelii*, and *B garinii*) cause the infection. Atypical forms of acrodermatitis chronica atrophicans under the clinical picture of anetoderma have to be considered.

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