Report

Evidence for *Borrelia burgdorferi* in morphea and lichen sclerosus

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Abstract

Background *Borrelia burgdorferi* (Bb) infection has been implicated in the development of morphea and lichen sclerosus; however, conflicting results have been reported with different investigational methods from different regions. We looked for evidence of Bb in patients with morphea and lichen sclerosus by polymerase chain reaction (PCR) analysis of of skin biopsy samples.

Methods Formalin-fixed, paraffin-embedded skin biopsy samples from 10 patients with morphea and 12 patients with lichen sclerosus were investigated by PCR analysis for the presence of Bb.

Results The presence of Bb DNA was demonstrated in three of 10 patients with morphea and six of 12 patients with lichen sclerosus by nested PCR.

Conclusions The data obtained in this study suggest that Bb may play a role in the etiopathogenesis of both morphea and lichen sclerosus at least in the western parts of Turkey.

Introduction

Morphea and lichen sclerosus are connective tissue disorders of unknown etiology,[1,2] that are characterized by inflammatory infiltrates in the initial stages, and thickened bundles of collagen in the later stages.[2,3] A familial occurrence has been described in isolated cases of morphea and lichen sclerosus. Also, in the etiopathogenesis, trauma, vaccination, radiotherapy, hormonal factors, and infections have been implicated.[1,2] In recent years, there have been some reports linking lichen sclerosus and morphea to *Borrelia burgdorferi* (Bb) infection, supporting a possible etiologic relationship between these two skin disorders.[4–12] This may be important, as the coexistence of morphea and lichen sclerosus has occasionally been reported.[6,13] On the other hand, controversy between the reports about the actual role of Bb in the etiopathogenesis of morphea and lichen sclerosus remains to be solved.[5,8–12,14–24]

Different direct and indirect investigational methods, including serologic tests, demonstration of spirochetes in tissues, culture of spirochetes, lymphocyte proliferation tests, and polymerase chain reaction (PCR), were used to detect Bb in morphea and lichen sclerosus patients;[5,8,9,25] however, reliable data could not be obtained due to technical limitations. The culture of Bb from tissue biopsies is generally unsuccessful, and microscopic detection of the spirochete is not sensitive.[25–27] Indirect methods, such as serology, are limited by low sensitivity and cross-reactions.[25,26,28] The PCR technique, which can amplify specific target DNA sequences one million times, has provided new possibilities for the detection of many microorganisms including Bb.[27,29–31] Recently, this technique has also been used for the determination of the role of Bb in the etiology of morphea and lichen sclerosus; nevertheless, the results were still controversial from different countries.[8,11,12,17–24] In this first study from Turkey, nested PCR was used to amplify selectively Bb specific gene from formalin-fixed, paraffin-embedded tissue biopsies obtained from patients with morphea and lichen sclerosus.

Materials and methods

Twenty two patients with morphea and lichen sclerosus from the western parts of Turkey were included in the study (Fig. 1). The mean age was 37.2 years (range, 7–68 years). The clinical diagnosis of morphea and lichen sclerosus was confirmed by

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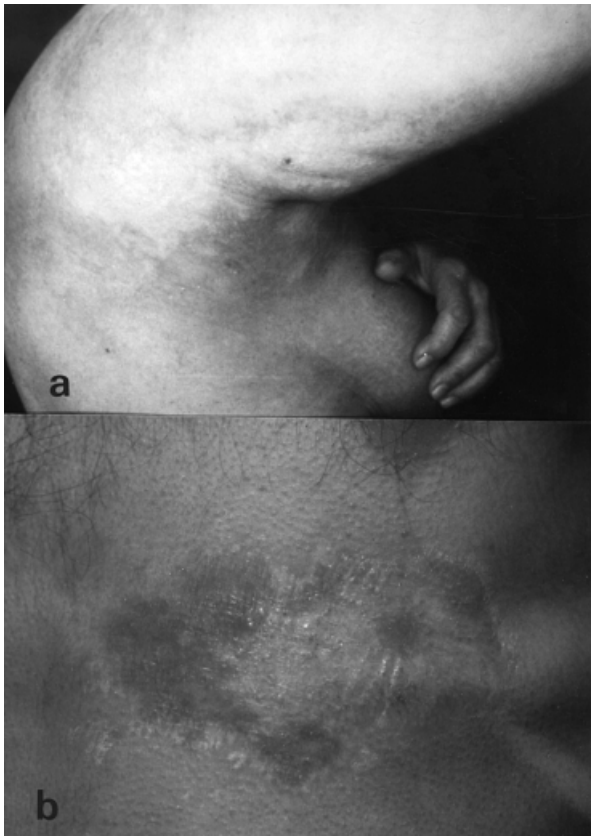

Figure 1 (a) Sclerotic morphea lesion. (b) Lichen sclerosus lesion showing follicular keratoses

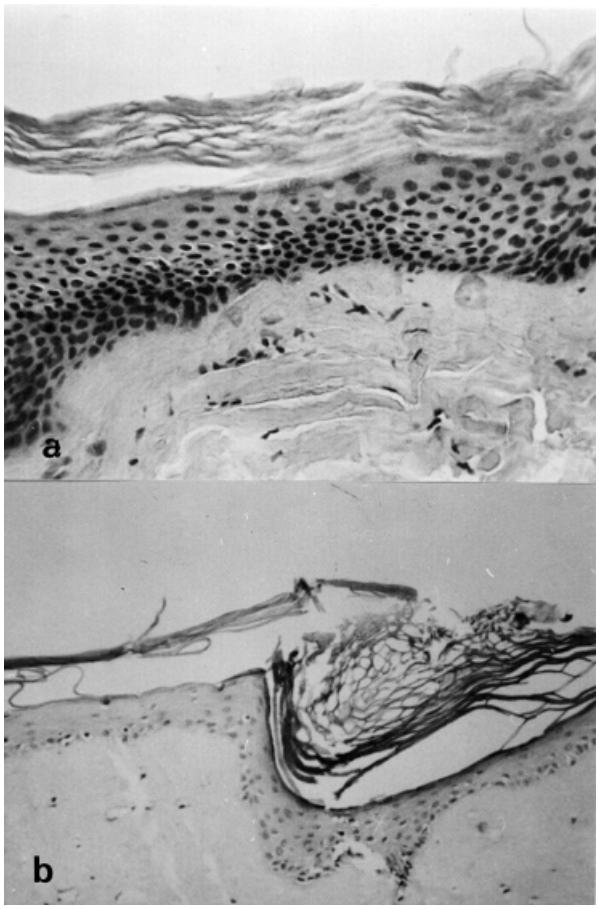

Figure 2 (a) Hyperkeratosis of the epidermis, increased pigmented melanocytes in the basal layer, and thickened collagen bundles in the superficial and reticular dermis of the morphea lesion (×200). (b) Atrophy of the epidermis, loss of rete ridges, and hydropic degeneration of the cells in the basal layer of the lichen sclerosus lesion (×100)

histologic criteria (Fig. 2). Two of 10 morphea patients also showed clinically lichen sclerosus-like lesions and three of 12 lichen sclerosus patients also showed clinically morphea-like lesions. Two patients with lichen sclerosus showed genital lesions. The onset of the clinical lesions was between 2 months and 6 years before the biopsies were performed. None of the patients had been specifically treated with antibiotics for suspected spirochetal infection before the skin biopsies were taken. Two patients with morphea remembered a tick bite. Ten patients (mean age, 41.1 years; 11–74 years) without any symptoms of Lyme disease and who did not have morphea or lichen sclerosus lesions were taken as the control group. After their skin biopsies had been taken, doxycycline therapy was given to all patients for 3 weeks and conflicting results were obtained. This study was approved by an ethics committee and the patients gave their informed consent.

DNA isolation

DNA extraction from formalin-fixed, paraffin-embedded tissue sections was performed as described with some modifications.[32] After deparaffinization and proteinase K digestion, supernatant was directly used as a template for PCR.

Primers

Bb European strain flagellin gene specific two primer sets designed by Melchers *et al.*[27] were used for PCR. The sequences for the first set of primers were: sense: 5′ GAT AAA AAC GAA GAT AAT CG; antisense: 5′ ACT AGG ATC TGT GGA TAT TC; they amplified a 356 base pair (bp) DNA fragment of the Bb European strain. The second set of primers which produced a 126 bp product were used for nested reaction. The sequences of the nested inner primers were: sense: 5′ CCA ACT TTA TCA AAT TCT GC; antisense: 5′ AGG ATC TAT TCC AAA ATC (Genemed Synthesis, Inc., USA).

PCR amplification and detection of sequence products

Bb specific target sequences were amplified in a 30 μL reaction mixture containing 10 × PCR buffer (Promega), 1.5 mM $MgCl_2$, 200 μM each of deoxyribose triphosphates, 20 pmol each of

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Table 1 The PCR results of the study patients with morphea and lichen sclerosus and of the control group

	Morphea patients	Lichen sclerosus patients	Control group
Positive PCR	3	6	0
Negative PCR	7	6	10
Total	10	12	10

primer, and 1.25 U Taq DNA polymerase (Promega). In the first step, the 356 bp fragment was amplified in a programmable heating block (PTC-100™, MJ Research, USA). After initial denaturation at 95 °C for 7 min, 35 cycles of PCR were performed: 1 min at 94 °C, 1 min at 37°C, and 1 min at 60 °C. Subsequently, 0.5 μL of this product was used as a template in the second PCR with nested primers in the same PCR mixture. Amplification of the 126 bp fragment was performed under the following conditions: 35 cycles of 1 min at 94 °C, 45 s at 50 °C, 1 min at 72 °C, and 7 min at 72 °C for final extension. For each PCR reaction, Bb European strain flagellin gene specific DNA obtained from Bb amplimer inserted InvitroGen TA-vector (pCR©2.1) (a generous gift of Willem J.G. Melchers, University Hospital Nijmegen, Nijmegen, Netherlands) was used as a positive control and distilled water as a negative control. We also used control tissue biopsies as negative control obtained from 10 patients with different diagnoses. PCR products were analyzed by ethidium bromide staining of agarose gel electrophoresis and visualized by UV light.

Results

A specific 126 bp amplification product was detected in three of 10 patients with morphea and six of 12 patients with lichen sclerosus (one showed genital lesions) by nested PCR (Table 1). In contrast, no specific amplification was seen in the 10 DNA samples obtained from control tissue biopsies and negative control (distilled water) (Fig. 3). The specific amplification was observed from DNA obtained by Bb amplimer inserted InvitroGen TA-vector (pCR©2.1).

Discussion

Lyme disease is a multisystem illness caused by the spirochete Bb. Cutaneous manifestations include "erythema migrans," "*Borrelia* lymphocytoma," and "acrodermatitis chronica atrophicans".[33] Various types of sclerotic and atrophic skin lesions have been reported in association with acrodermatitis chronica atrophicans, lymphocytoma, and Lyme arthritis.[33–35]

Since the relationship between Bb and morphea was first reported by Aberer *et al.*[4] from Austria, many investigations have been conducted to look for a possible association between this microorganism and morphea or lichen sclerosus. Different diagnostic approaches have been used and conflicting results have been reported.[4,5,7–12,14–23] Current laboratory diagnosis consists of a variety of serologic assays for detection of antibodies to Bb which can be achieved within days;[26] however, this approach is limited by low sensitivity (false negativity), low specificity (false positivity), significant intra- and interlaboratory variability, and by the fact that only the patient's response, not the spirochete itself, is recognized.[19,26,28] The diagnosis of Lyme disease based on the culture of the spirochete is less than ideal because of the complexity, the time required,[31] and the high number of false negatives.[11] Also, false negative results have been reported frequently with lymphocyte proliferation tests.[36] Recently, attention has focused on the amplification of DNA with PCR as an aid in diagnosis.[11,26,27] PCR is being increasingly used to detect even small numbers of infectious agents that may be difficult or impossible to grow in culture.[8,11] The potential sensitivity, specificity, and rapid return of the results of PCR make it a particularly appealing procedure for detecting Bb,[31,37] The PCR technique was also used to investigate Bb in patients with morphea and lichen sclerosus. The results of these studies are listed in Table 2.[8,11,12,17–24]

PCR analysis has been used to investigate the roles of many infectious microorganisms in the pathogenesis of some cutaneous diseases. Several dermatologic diseases and associated microorganisms, linked by PCR, have been reported: erythema induratum of Bazin, papulonecrotic

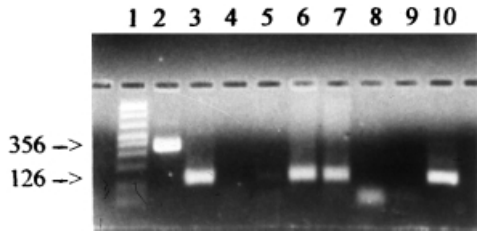

Figure 3 Nested PCR detection of *Borrelia burgdorferi* in Lyme patients: lane 1, amplisize DNA size standard (50–2000 bp ladder); lane 2, first PCR product of positive control (356 bp); lane 3, second PCR product of positive control (126 bp); lane 4, first PCR product of negative control; lane 5, second PCR product of negative control; lane 6, second PCR product of patient 1 (*Borrelia burgdorferi* positive); lane 7, second PCR product of patient 3 (*Borrelia burgdorferi* positive); lane 8, second PCR product of patient 2 (*Borrelia burgdorferi* negative); lane 9, second PCR product of patient 4 (*Borrelia burgdorferi* negative); lane 10, second PCR product of patient 7 (*Borrelia burgdorferi* positive)

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Table 2 The results of studies investigating *Borrelia burgdorferi* in morphea and lichen sclerosus patients by polymerase chain reaction[8,11,12,17–24]

	Country	Year	Diagnosis and number of patients	Positive PCR results
8	Germany	1993	M: 9 patients LS: 6 patients	M: 9 patients LS: 6 patients
17	Netherlands	1993	M: 12 patients	None
22	Finland	1994	M: 7 patients	None
21	Germany	1995	M: 30 patients	None
19	USA	1995	M: 30 patients	None
18	USA	1995	M: 20 patients LS: 10 patients	None
11	Italy	1996	M: 10 patients	M: 6 patients
20	USA	1996	M: 28 patients LS: 7 patients	None
12	Germany	1997	M: 4 patients LS: 10 patients	M: 3 patients LS: 2 patients
12	Japan	1997	M: 5 patients LS: 3 patients	M: 2 patients
12	USA	1997	M: 10 patients LS: 21 patients	None
24	USA	1997	M: 13 patients LS: 13 patients M+LS: 1 patient	None
23	Spain	1997	M: 6 patients LS: 8 patients	None
This study	Turkey	1998	M: 10 patients LS: 12 patients	M: 3 patients LS: 6 patients

M, morphea; LS, lichen sclerosus; PCR, polymerase chain reaction.

tuberculid, sarcoidosis, and *Mycobacterium tuberculosis*;[38–40] alopecia areata and cytomegalovirus;[41] erythema multiforme and herpes simplex;[42,43] Kaposi's sarcoma and human herpes virus type 8;[44] lichen planus and hepatitis C virus;[45] oral lichen planus and human papillomavirus;[46] pityriasis rosea and human herpes virus type 7.[47]

PCR sensitivity was higher when fresh frozen skin specimens were used than when formalin-fixed, paraffin-embedded tissues were employed as specimens.[37] In our study, formalin-fixed, paraffin-embedded tissues were investigated. Conflicting results have been obtained from different geographic regions concerning positive results for Bb in morphea and lichen sclerosus patients.[5,8–11,14–23] While positive results were reported from Austria, Switzerland, Germany, Italy and Japan, negative results were reported from Denmark, France, Netherlands, Spain, Finland, and the USA.[8–12,17-24] To explain these conflicting results, differences in the prevalence of antibodies in the general population or differences between *Borrelia* strains isolated in Europe and the USA have been evoked.[16] Recently, three separate genospecies have been identified: *B. garinii*, *B. afzelii*, *B. burgdorferi sensu stricto*. All three strains are present in Europe, but only *B. burgdorferi sensu stricto* is present in the USA.[34] Also, clinical variations of Lyme disease have been detected among different regions and this was correlated with different strains of Bb.[48,49] In North America, Bb DNA was undetectable in patients with morphea. These negative findings might be because *B. burgdorferi sensu stricto*, the predominant strain in North America, has never been found in association with late dermatologic manifestations of Lyme disease.[37] It has been suggested that only infection with certain strains of Bb would lead to the development of morphea; this may also be true for lichen sclerosus. This may explain the conflicting data obtained from different regions.[34] Also, the genetic background was emphasized as being important for the immune response to Bb. Thus, it has been proposed that the middle European population might have a common genetic predisposition for developing sclerotic skin lesions during a Bb infection.[48] Although PCR sensitivity is lower for paraffin-embedded tissues, the high positive results in our study suggest that Bb may play a role in the pathogenesis of both morphea and lichen sclerosus, at least in the western parts of Turkey.

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References

1 Rowell NR, Goodfield MJD. The connective tissue diseases. In: Champion RH, Burton JL, Burns DA, Breathnach SM, eds. *Textbook of Dermatology*. Oxford: Blackwell Sciences, 1998: 2437–2575.

2 Braun-Flaco O, Plewig G, Wolff HH, Winkelmann RK. *Dermatology*. Berlin: Springer-Verlag, 1991: 533–585.

3 Arnold HL, Odom RB, James WD. *Andrews' Diseases of the Skin*. Philadelphia: W.B. Saunders, 1990: 176–181, 595–597.

4 Aberer E, Neumann R, Stanek G. Is localised scleroderma a borrelia infection? *Lancet* 1985: **2:** 278.

5 Aberer E, Stanek G, Ertl M, Neumann R. Evidence for spirochetal origin of circumscribed scleroderma (morphea). *Acta Derm Venereol (Stockh)* 1987: **67: 225–231.**

6 Meffert JJ, Davis BM, Grimwood RE. Lichen sclerosus. *J Am Acad Dermatol* 1995; **32:** 393–416.

7 Abele DC, Anders KH. The many faces and phases of borreliosis II. *J Am Acad Dermatol* 1990; **23:** 401–410.

8 Schempp C, Bocklage H, Lange R. *et al*. Further evidence for Borrelia burgdorferi infection in morphea and lichen sclerosus et atrophicus confirmed by DNA amplification. *J Invest Dermatol* 1993; **100:** 717–720.

9 Buechner SA, Winkelmann RK, Lautenschlager S, *et al*. Localized scleroderma associated with Borrelia burgdorferi infection. *J Am Acad Dermatol* 1993; **29:** 190–196.

10 Breier F, Klade H, Stanek G, *et al*. Lymphoproliferative responses to Borrelia burgdorferi in circumscribed scleroderma. *Br J Dermatol* 1996; **134:** 285–291.

11 Trevisan G, Stinco G, Nobile C, *et al*. Detection of Borrelia burgdorferi in skin biopsies from patients with morphea by polymerase chain reaction. *J Eur Acad Dermatol Venereol* 1996; **6:** 15–19.

12 Fujiwara H, Fujiwara K, Hashimoto K, *et al*. Detection of Borrelia burgdorferi DNA (B. garinii or B. afzelii) in morphea and lichen sclerosus et atrophicus tissues of German and Japanese but not of US patients. *Arch Dermatol* 1997; **133:** 41–44.

13 Shono S, Imura M, Ota M, *et al*. Lichen sclerosus et atrophicus, morphea, and coexistence of both diseases. Histological studies using lectins. *Arch Dermatol* 1991; **127:** 1352–1356.

14 Hansen K, Serup J, Hoybye S. Antibodies to Borrelia burgdorferi and localised scleroderma. *Lancet* 1987; **1:** 682.

15 Lecerf V, Bagot M, Revuz J, *et al*. Borrelia burgdorferi and localized scleroderma. *Arch Dermatol* 1989; **125:** 297.

16 Vaillant L, Goudeau A. Localized scleroderma is not a Borrelia burgdorferi infection in France. *Dermatology* 1992; **184:** 286.

17 Meis JF, Koopman R, van Bergen B, *et al*. No evidence for a relation between Borrelia burgdorferi infection and old lesions of localized scleroderma (morphea). *Arch Dermatol* 1993; **129:** 386–387.

18 Dillon WI, Saed GM, Fivenson DP. Borrelia burgdorferi DNA is undetectable by polymerase chain reaction in skin lesions of morphea, scleroderma, or lichen sclerosus et atrophicus of patients from North America. *J Am Acad Dermatol* 1995; **33:** 617–620.

19 Fan W, Leonardi CL, Penneys NS. Absence of Borrelia burgdorferi in patients with localized scleroderma (morphea). *J Am Acad Dermatol* 1995; **33:** 682–684.

20 De Vito JR, Merogi AJ, Vo T, *et al*. Role of Borrelia burgdorferi in the pathogenesis of morphea/scleroderma and lichen sclerosus et atrophicus: a PCR study of thirty-five cases. *J Cutan Pathol* 1996; **23:** 350–358.

21 Wienecke R, Schlupen EM, Zochling N, *et al*. No evidence for Borrelia burgdorferi-specific DNA in lesions of localized scleroderma. *J Invest Dermatol* 1995; **104: 23–26.**

22 Ranki A, Aavik E, Peterson P, *et al*. Successful amplification of DNA specific for Finnish Borrelia burgdorferi isolates in erythema chronicum migrans but not in circumscribed scleroderma lesions. *J Invest Dermatol* 1994; **102:** 339–345.

23 Alonso-Llamazares J, Persing DH, Anda P, *et al*. No evidence for Borrelia burgdorferi infection in lesions of morphea and lichen sclerosus et atrophicus in Spain. *Acta Derm Venereol (Stockh)* 1997; **77:** 299–304.

24 Colomé-Grimmer MI, Payne DA, Tyring SK, Sánchez RL. Borrelia burgdorferi DNA and Borrelia hermsii DNA are not associated with morphea or lichen sclerosus et atrophicus in the southwestern United States. *Arch Dermatol* 1997; **133:** 1174.

25 Berg D, Abson KG, Prose NS. The laboratory diagnosis of Lyme disease. *Arch Dermatol* 1991; **127:** 866–870,.

26 Wienecke R, Neubert U, Volkenandt M. Molecular detection of Borrelia burgdorferi in formalin-fixed, paraffin-embedded lesions of Lyme disease. *J Cutan Pathol* 1993; **20:** 385–388.

27 Melchers W, Meis J, Rosa P, *et al*. Amplification of Borrelia burgdorferi DNA in skin biopsies from patients with Lyme disease. *J Clin Microbiol* 1991; **29:** 2401–2406.

28 Moter SE, Hofmann H, Wallich R, *et al*. Detection of Borrelia burgdorferi sensu lato in lesional skin of patients with erythema migrans and acrodermatitis chronica atrophicans by ospA-specific PCR. *J Clin Microbiol* 1994; **32:** 2980–2988.

29 Rosa PA, Schwan TG. A specific and sensitive assay for the Lyme disease spirochete Borrelia burgdorferi using the polymerase chain reaction. *J Infect Dis* 1989; **160:** 1018–1029.

30 Rosa PA, Hogan D, Schwan TG. Polymerase chain reaction analyses identify two distinct classes of Borrelia burgdorferi. *J Clin Microbiol* 1991; **29:** 524–532.

31 Schwartz I, Wormser GP, Schwartz JJ, *et al*. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans lesions. *J Clin Microbiol* 1992; **30:** 3082–3088.

32 Cingöz S, Yörükoğlu K, Atabey N, *et al*. DNA isolation for polymerase chain reaction from formalin fixed and

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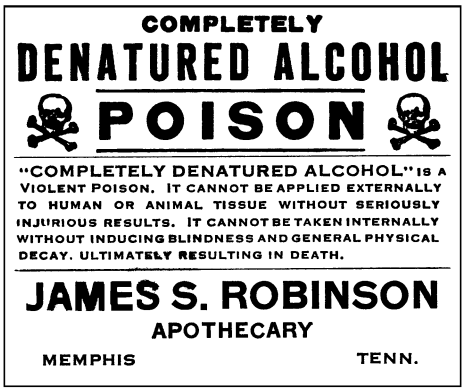
paraffin embedded tissues. *Tr J Medical Science* 1995; **24:** 259–262.
33 Malane MS, Grant-Kels JM, Feder HM, Luger SW. Diagnosis of Lyme disease based on dermatologic manifestations. *Ann Intern Med* 1991; **114:** 490–498.
34 Trevisan G, Rees DH, Stinco G. Borrelia burgdorferi and localized scleroderma. *Clin Dermatol* 1994; **12:** 475–479.
35 Hovmark A. Role of Borrelia burgdorferi in lymphocytomas and sclerotic skin lesions. *Clin Dermatol* 1993; **11:** 363–367.
36 Dressler F, Yoshinari NH, Steere AC. The T cell proliferative assay in the diagnosis of Lyme disease. *Ann Intern Med* 1991; **115:** 533–539.
37 Schmidt BL. PCR in laboratory diagnosis of human Borrelia burgdorferi infections. *Clin Microbiol* 1997; **10:** 185–201.
38 Schneider JW, Jordaan HF, Geiger DH, *et al.* Erythema induratum of Bazin. A clinicopathological study of 20 cases and detection of Mycobacterium tuberculosis DNA in skin lesions by polymerase chain reaction. *Am J Dermatopathol* 1995; **17:** 350–356.
39 Victor T, Jordaan HF, Van Niekerk DJ, *et al.* Papulonecrotic tuberculid: identification of Mycobacterium tuberculosis DNA by polymerase chain reaction. *Am J Dermatopathol* 1992; **14:** 491–495.
40 Fidler HM, Rook GA, Johnson NM, McFadden J. Mycobacterium tuberculosis DNA in tissue affected by sarcoidosis. *Br Med J* 1993; **306:** 546–549.
41 Skinner RB Jr, Light WH, Bale GF, *et al.* Alopecia areata and presence of cytomegalovirus DNA. *JAMA* 1995; **273:** 1419–1420.
42 Darragh TM, Egbert BM, Berger TG, Yen TS. Identification of herpes simplex virus DNA in lesions of erythema multiforme by the polymerase chain reaction. *J Am Acad Dermatol* 1991; **24:** 23–26.
43 Aslanzadeh J, Helm KF, Espy MJ, *et al.* Detection of HSV-specific DNA in biopsy tissue of patients with erythema multiforme by polymerase chain reaction. *Br J Dermatol* 1992; **126:** 19–23.
44 Jensen P, Huang YQ, Li JJ, *et al.* Herpes virus like DNA (HHV-8) in immunosuppressive therapy related, HIV related and classical Kaposi's sarcoma in Norwegian patients. *Acta Derm Venereol* 1998; **78:** 205–206.
45 Imhof M, Popal H, Lee JH, *et al.* Prevalence of hepatitis C virus antibodies and evaluation of hepatitis C virus genotypes in patients with lichen planus. *Dermatology* 1997; **195:** 1–5.
46 Jontell M, Watts S, Wallstrom M, *et al.* Human papilloma virus in erosive oral lichen planus. *J Oral Pathol Med* 1990; **19:** 273–277.
47 Drago F, Ranieri E, Malaguti F, *et al.* Human herpesvirus 7 in patients with pityriasis rosea. Electron microscopy investigations and polymerase chain reaction in mononuclear cells, plasma and skin. *Dermatology* 1997; **195:** 374–378.
48 Aberer E, Klade H. Reply. *Dermatology* 1992; **184:** 287–288.
49 Asbrink E, Hovmark A. Classification, geographic variations, and epidemiology of Lyme borreliosis. *Clin Dermatol* 1993; **11:** 353–357.



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