SCLEROSING AND GRANULOMATOUS SKIN LESIONS IN BORRELIA BURGDORFERI INFECTION

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ABSTRACT

The pathogenetic linkage between *Borrelia burgdorferi* and the cutaneous manifestations of Lyme Borreliosis, i.e. erythema migrans, borrelial lymphocytoma and acrodermatitis chronica atrophicans is well established. Moreover, in three other dermatoses - circumscribed scleroderma, lichen sclerosus et atrophicus and granuloma annulare - laboratory findings indicated that these diseases might be associated with a *Borrelia burgdorferi* infection. In particular, cellular or humoral immune responses to *Borrelia burgdorferi*, as measured by lymphocyte proliferation tests or by immunoblot investigations, respectively, were detected in some circumscribed scleroderma patients. In sporadic cases *Borrelia burgdorferi* was even isolated from lesional skin of patients with circumscribed scleroderma or granuloma annulare. Recently developed molecular biological techniques provide tools to detect *Borrelia burgdorferi*-DNA in several tissues and body fluids. Particularly the excretion of *borrelia*-DNA in the urine, detected by PCR, was proven to be a useful and reliable laboratory parameter for the clinical correlation of disease activity in erythema migrans and acrodermatitis chronica atrophicans. Therefore, we investigated the excretion of *Borrelia burgdorferi*-DNA in the urine of patients with circumscribed scleroderma, lichen sclerosus et atrophicus and granuloma annulare by amplification of a 276 bp flagellin gene segment using a nested PCR technique. Surprisingly, PCR was positive in 61% of patients with circumscribed scleroderma, 68% of patients with lichen scleroses et atrophicus and 61% of patients with granuloma annulare. These data provide evidence for the presence of a systemic *Borrelia burgdorferi* infection in a subset of patients with these dermatoses and open up a field to develop new concepts in the treatment of these patients.

KEY WORDS

Borrelia burgdorferi, circumscribed scleroderma, lichen sclerosus et atrophicus, granuloma annulare, immunoblot-investigation, cellular immune response, polymerase chain reaction, urine

INTRODUCTION

The clinical spectrum of systemic and localized manifestations of Lyme Borreliosis (LB) increases steadily but has not yet been defined precisely. Beside the classical cutaneous manifestations of LB, erythema migrans (EM), borrelial lymphocytoma (BL) and acrodermatitis chronica atrophicans (ACA), further dermatoses, such as circumscribed scleroderma (morphea) (CS), lichen sclerosus et atrophicus (LSA)

and granuloma annulare (GA) have been linked to a *Borrelia burgdorferi* (*Bb*) infection (1,2). Upon staining with anti-*Bb* polyclonal antibodies, *borrelia*-like organisms were detected in the skin in these three dermatoses by immunoperoxidase methods (3,4). Cultivation of *Bb* was reported in sporadic cases of CS and GA (2,5). In earlier studies 30 patients with different types of CS were tested for serum antibodies against *Bb* determined by ELISA and Westernblot analysis (1,6). *Bb* B 31 sensu stricto was used as an antigen for SDS-electrophoresis. Forty-six percent of CS patients were IgG seropositive based on ELISA. Westernblot examinations confirmed the ELISA results in 10 out of 25 patients showing an antibody profile which can also be seen in the course of LB. The *Bb* seropositive patients could mostly be associated with a symmetric-plaque and linear-systemized type of morphea. In further experiments, the cellular immune response to *Bb* was investigated (7). When isolated peripheral blood mononuclear cells from CS patients were incubated with *Bb*, genospecies *garinii* (*Bg*), proliferation of these cells was induced in 11 (5 seropositive and 6 seronegative) out of 39 patients.

More recently, molecular biological techniques, such as PCR, made the detection of *Bb*-DNA in the skin and body fluids possible (8,9). As demonstrated in several studies, *Bb* organisms have a high affinity to the urine bladder tissue and *Bb*-antigens and DNA are secreted in the urine during the course of LB (10-12).

In this paper we focuse on the correlation between different in vitro methods for laboratory diagnosis of *Bb* infection in CS, LSA, and GA. The data presented here were partly published previously (1,7,13,14) and are reviewed in this paper.

MATERIALS AND METHODS

LYMPHOCYTE TRANSFORMATION TESTS AND IMMUNOBLOTS IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA

In a first series of experiments lymphocyte transformation tests (LTT) were performed in 39 patients with different types of CS as previously described (7). In brief, 10^5 peripheral blood mononuclear cells of patients were incubated with 10^6 *Bg* organisms for 5 days. Eighteen hours before harvesting, ^3H thymidine was added and its uptake by mononuclear cells measured in a liquid scintillation counter. The counts were expressed as distintegrations per minute and a stimulation index (SI) was calculated. Control experiments were carried out in 6 healthy individuals, 16 patients with non-LB-associated skin diseases and in patients with EM and ACA.

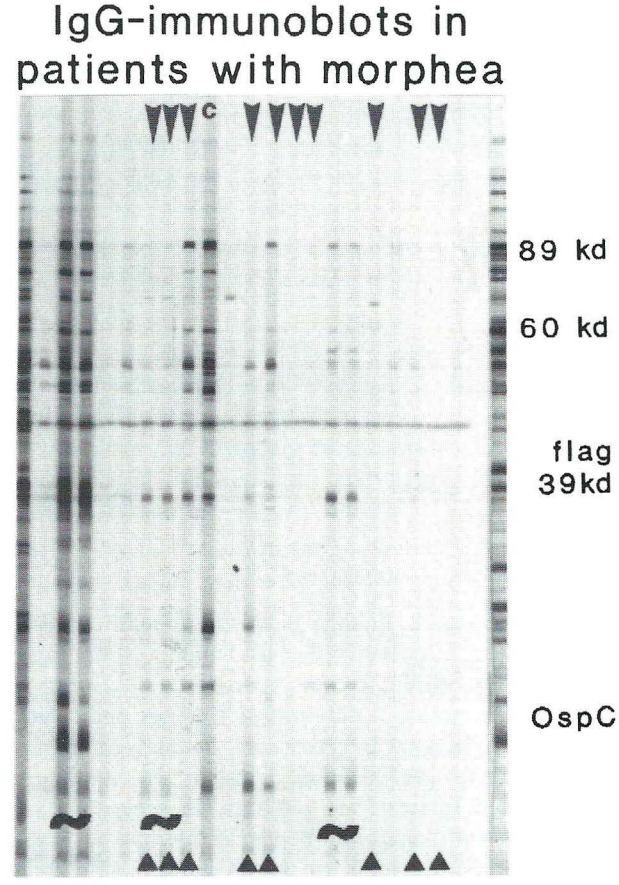

Fig. 1. IgG-immunoblots of sera from 18 patients with circumscribed scleroderma.
Right lane: Molecular weight standards; C: Control serum of an acrodermatitis chronica atrophicans patient. Arrows at the top indicate the patient's sera with positive lymphocyte transformation tests; arrows at the bottom indicate positive immunoblots in lymphocyte transformation test positive patients. Curved lines indicate paired sera taken from circumscribed scleroderma patients before and after antibiotic treatment.

Immunoblot (surfblot, IDEA Scientific Company, Minneapolis, MN, U.S.A.) (IB) studies were performed from sera of 18 patients with CS (unpublished data). In 3 of these patients samples before and after treatment were examined. Paired samples were examined for LTT and IB studies in 14 patients. A membrane fraction of *Bb*, genospecies *afzelii* (*Ba*), was applied on a SDS - polyacrylamid

electrophoresis gel. After separation, proteins were transferred to nitrocellulose membranes. The patients' sera, in a dilution of 1:250 for IgG and 1:10 for IgM detection, were applied to these strips and the bound antibodies detected by alkaline phosphatase conjugated goat anti-human immunoglobulin G or M. To determine relevant antigens, sera obtained from patients with the acute, disseminated and chronic phase of LB as well as from patients with ACA and healthy blood donors were also tested (data not shown). Based on the statistical evaluation of the results obtained from patients and healthy blood donors seropositivity criteria of four or more reacting bands for IgG and three or more bands for IgM were established.

PCR INVESTIGATIONS IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA, LICHEN SCLEROSUS ET ATROPHICUS AND GRANULOMA ANNULARE

In a further series of experiments the urine excretion of *Bb*-DNA was investigated in 63 patients, 31 with CS, 19 with LSA and 13 patients with GA (unpublished data). By means of a nested PCR, a 276 bp flagellar gene sequence of *Bb*-DNA was amplified as previously described (9). In addition, antibodies against *Bb* were investigated in the sera of these 63 patients by ELISA (7).

CULTURE OF *BORRELIA BURGDORFERI* IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA AND LICHEN SCLEROSUS ET ATROPHICUS

Punch biopsies (4mm) were taken from the expanding border of CS or LSA skin lesions and cultured in a Barbour-Stoenner-Kelly medium (6).

RESULTS

CORRELATION OF POSITIVE LYMPHOCYTE TRANSFORMATION TESTS AND IMMUNOBLOTS IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA

Peripheral blood mononuclear cells from 11 out of 39 patients (28%) showed an elevated SI when exposed to *Bb* antigens as compared to samples of healthy control persons. 4-20 bands were detected in blots of CS patients, whereas IBs presented more than 20 bands in ACA. IBs were positive in 13 out of 18 CS serum samples (72%), in 3 patients for both IgM and IgG, in 9 patients for IgG and in 1 patient for IgM antibodies only. In 3 patients 2 samples were examined before and after treatment with almost identical bands. Seven out of 14 patients (50%) where LTTs and IBs were performed simultaneously, revealed positive results in both test systems (Figs. 1,2). The type of CS in these patients belonged to a symmetric-plaque type. Moreover, 4 patients were positive in LTTs and 3 in IBs only.

NESTED PCR FOR DETECTION OF *BORRELIA BURGDORFERI* FLAGELLIN-GENE FRAGMENTS AND SEROLOGICAL FINDINGS AS DETERMINED BY ELISA IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA, LICHEN SCLEROSUS ET ATROPHICUS AND GRANULOMA ANNULARE

Whereas in patients with EM, the early phase of LB, *borrelia*-DNA is excreted in the urine in about

Table. Culture of Borrelia burgdorferi from skin biopsies of patients with circumscribed scleroderma and lichen sclerosus et atrophicus.

Pat.	Age	Sex	Diagnosis	Clinical Type	*Borrelia* genospecies
1	49	M	CS[1]	linear-systemized	*B. burgdorferi* sensu lato
2	45	M	CS[1]	linear-systemized	*B. afzelii*
3	55	M	CS[1]	symmetric-plaque	*B. afzelii*
4	69	F	LSA[2]	symmetric-plaque and gential area	*B.afzelii*

1 = circumscribed scleroderma
2 = lichen sclerosus et atrophicus

90% (9), 19 out of 31 (61%) patients with CS were positive by PCR. Four of these patients were also seropositive, whereas 3 other patients were seropositive but negative in their urine PCR. In 9 patients no *Bb*-specific laboratory data could be evaluated.

LSA patients tested PCR positive in 13/19 (68%), 7 out of these 13 patients were also seropositive, 1 additional patient was seropositive but PCR-negative.

In GA *borrelia*-DNA excretion was detectable in 8/13 (61%) patients; only one of these patients was also seropositive.

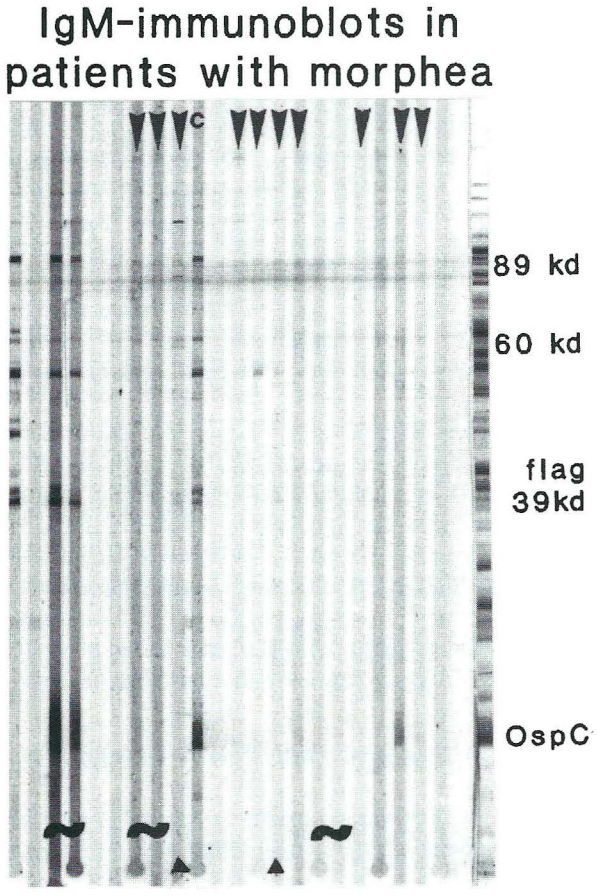

Fig. 2. IgM-immunoblots of sera from 18 patients with circumscribed scleroderma.
Right lane: Molecular weight standards; C: Control serum of an acrodermatitis chronica atrophicans patient. Arrows at the top indicate the patient's sera with positive lymphocyte transformation tests; arrows at the bottom indicate positive immunoblots in lymphocyte transformation test positive patients. Curved lines indicate paired sera taken from circumscribed scleroderma patients before and after antibiotic treatment.

CULTURE OF *BORRELIA BURGDORFERI* IN PATIENTS WITH CIRCUMSCRIBED SCLERODERMA AND LICHEN SCLEROSUS ET ATROPHICUS

As previously published, *Bb* was cultivated from a patient with a linear-systemized type of CS (6). This *borrelia* isolate was identified as *Bb* by immunofluorescence-methods using monoclonal antibodies, but could not be further typed. In following culture trials *Bb*, genospecies *Ba* as identified by serotyping with monoclonal antibodies and by PCR-methods (7), could be isolated from two further CS and one LSA patient (unpublished data, see Table).

DISCUSSION

The etiology of certain dermatoses affecting the connective tissue, such as CS, LSA and GA, is still unknown. CS, which can be induced by drugs, mechanical and immunological factors, involves the dermis, subcutaneous fat and fascia leading to fibrosis of collagenous tissue (15). LSA is also a disease of unknown origin. It can be observed in genital and extragenital skin (16). The coexistence of CS and LSA in several patients favours the assumption that both conditions may be caused or triggered by analogous agents. Many reports have focused on their possible relationship to a *Bb* infection, however, contradicting data was published (1,17). Based on serological and molecular biological studies, suspected *borrelia*-induced CS and LSA have only been reported in European countries (18,19,20). The association with a *Bb* infection was more likely to be observed in the symmetric-plaque and linear-systemized type of morphea as evaluated by IB and LTT investigations (1,7). In recent studies patients with atrophodermia Pasini-Pierini, the atrophic variant of CS, were mostly seronegative but showed positive LTT and positive *Bb*-DNA in their urine in single cases (7, unpublished data).

When comparing laboratory data of patients with different types of CS showing humoral or cellular immunity to *Bb*, 7 out of 14 patients (50%) were positive in both tests. Whereas LTTs are not performed routinely, IB-investigations are well established for the diagnosis of LB. Similar to serological testing, however, the standardization of methods as well as the standardization of interpreting results have not yet been achieved. The main reason for this fact is the heterogeneity of *borrelia* strains and the variety of antigen expression which is elicited in the different manifestations of LB (21). Currently,

the Centers for Disease Control and Prevention are developing criteria for interpretation of Western blot results (22).

Isolation of *Bb* from affected tissue is the most characteristic evidence and proof of infection. Three cases have been published so far where *Bb* was cultivated from a skin biopsy of CS. According to the clinical aspect, one patient was suffering from a linear-systemized type of CS (6), the two other reported patients from a large, partly symmetric-plaque type of CS, one of which with coexisting LSA (5). It is interesting to note that in our studies, a further culture-positive patient showed a linear-systemized type of CS, one patient a symmetric-plaque type on the trunk and an additional patient symmetric patches of LSA on the trunk. These findings, together with the high excretion rate of *Bb*-DNA in the urine, and the improvement of CS and LSA in some patients due to betalactam antibotics, underline the pathogenetic role of *Bb* in a subset of patients.

GA was said to be caused by mechanical, autoimmune and metabolic factors (23-25). In addition, infectious agents, such as viruses, were reported as pathogens. The development of GA was also observed after insect bites (23). In the literature *Bb* was isolated from a GA skin lesion of a patient with a history of a tick bite, arthralgias, fatigue, headache and seroconversion during the course of the disease (2). The isolated strain was of the Osp A serotype 2 (*Ba*) (26). Intravenous treatment with ceftriaxone resulted in a remission of the clinical symtoms and also of GA 5 months after treatment. In our study a high percentage of patients with GA (61%) showed *Bb*-DNA excretion in the urine. An association with arthralgias, and a tick or insect bite was also reported by PCR-positive patients.

In summary, we have shown that various laboratory data point to a *Bb* infection in a considerable number of patients with CS, LSA and GA. In the future, more sensitive laboratory studies are needed to confirm these findings and to explore the understanding of pathogenetic linkages between *Bb* and sclerosing and granulomatous dermatoses.

REFERENCES

1. Aberer E, Klade H, Stanek G, Gebhart W. *Borrelia burgdorferi* and different types of morphea. Dermatologica 1991; 181: 145-54.

2. Strle F, Preac-Mursic V, Ruzic E et al. Isolation of *Borrelia burgdorferi* from a skin lesion in a patient with granuloma annulare. Infection 1991; 19: 351-52.

3. Aberer E, Stanek G. Histological evidence for spirochetal orgin of morphea and lichen sclerosus et atrophicus. Am J Dermatopathol 1987; 9: 374-79.

4. Aberer E, Mainitz M, Neumann R, Stanek G. Immunoperoxidase staining of spirochetes in borrelial skin diseases. Ann NY Acad Sci 1988; 539: 362-64.

5. Weber K, Preac-Mursic V, Reimers CD. Spirochetes isolated from two patients with morphea. Infection 1988; 16: 25-26.

6. Aberer E, Stanek G, Ertl M, Neumann R. Evidence for spirochetal origin of circumscribed scleroderma (morphea). Acta Derm Venereol (Stockh) 1987; 67: 225-31.

7. Breier F, Klade H, Stanek G et al. Lymphoproliferative responses to *Borrelia burgdorferi* in circumscribed scleroderma. Br J Derm 1996; 134: 285-91.

8. Wienecke R, Zoechling N, Neubert U et al. Molecular subtyping of *Borrelia burgdorferi* in erythema migrans and acrodermatitis chronica atrophicans. J Invest Dermatol 1994; 103: 19-22.

9. Schmidt B, Aberer E, Stockenhuber C et al. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in the urine and breast milk of patients with Lyme Borreliosis. Diagn Microbiol Infect Dis 1995; 21: 121-28.

10. Aberer E, Neumann R, Lubec G. Acrodermatitis chronica atrophicans in association with lichen sclerosus et atrophicans: tubulo-interstitial nephritis and urinary excretion of spirochete-like organisms. Acta Derm Venerol (Stockh) 1987; 67: 62-65.

11. Dorward DW, Schwan TG, Garon CF. Immune capture and detection of *Borrelia burgdorferi* antigens in urine, blood, or tissues from infected ticks, mice, dogs and humans. J Clin Microbiol 1991: 29; 1162-70.

12. Hyde FW, Johnson RC, White TJ, Shelburne CE. Detection of antigens in urine of mice and humans infected with *Borrelia burgdorferi*, etiologic agent of Lyme disease. J Clin Microbiol 1989; 27: 58-61.

13. Aberer E, Breier F, Klade H. Sclerotic skin lesions in *Borrelia burgdorferi* infections. Dermatology 2000, Vienna, 18.-21.5.1993 (Abstract).

14. Aberer E, Schmidt B, Luger A. Urinary excretion of *Borrelia burgdorferi* DNA in patients with Lyme Borreliosis. Arch Derm Res 1994; 286: 222 (Abstract).

15. Rosenwasser TA, Eisen AZ. Scleroderma. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al. (eds.). Dermatology in General Medicine. Mc Graw Hill, New York, 1993: 2156-67.

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

17. Fan W, Leonardi CL, Penney NS. Absence of *Borrelia burgdorferi* in patients with localized scleroderma (morphea). J Am Acad Dermatol 1995; 33: 682-84.

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: 617-20.

19. 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: 717-20.

20. Weidenthaler B, Roux M, Moter SE et al. Sclerodermiform skin lesions in infection with *Borrelia burgdorferi*. Use of polymerase chain reaction for diagnosis. Hautarzt 1994; 45: 171-75.

21. Dressler F, Whalen JA, Reinhardt BC, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis 1993; 167: 392-400.

22. Centers for Disease Control and Prevention; Association of State and Territorial Public Health Laboratory Directors. Proceedings of the Second National Conference on Serologic Diagnosis of Lyme Disease. Dearborne, MI 1994.

23. Harth W, Richard G. Retinoide in der Therapie des Granuloma anulare disseminatum. Hautarzt 1993; 44: 693-98.

24. Tada J, Seno A, Ueda M et al. Association of generalized granuloma annulare with autoantibodies. J Dermatol 1993; 20: 293-97.

25. Kallionen M, Sandberg M, Kinnunen T, Oikarinen A. Collagen synthesis in granuloma annulare. J Invest Dermatol 1992; 98: 463-68.

26. Wilske B, Preac-Mursic V, Göbel UB et al. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. J Clin Microbiol 1993; 31: 340-50.

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