

Granulomatous Cheilitis and *Borrelia burgdorferi*

Polymerase Chain Reaction and Serologic Studies in a Retrospective Case Series of 12 Patients

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Background: Granulomatous cheilitis (GC) is a chronic granulomatous inflammation of the lips of unknown etiology, which may be associated with peripheral facial nerve paralysis and/or lingua plicata (Melkersson-Rosenthal syndrome [MRS]). *Borrelia burgdorferi* is a spirochete that causes Lyme borreliosis, a multisystemic infectious disease with frequent occurrence of facial nerve paralysis. An etiologic role of *B burgdorferi* in various granulomatous diseases has been suggested. The present study was performed to examine a possible causative role of *B burgdorferi* for GC/MRS by *B burgdorferi*-specific polymerase chain reaction analysis of biopsy specimens from affected lip tissue and determination of *B burgdorferi* IgG and IgM serum antibodies using enzyme-linked immunosorbent assay and immunoblot tests.

Observations: We examined a retrospective case series of 12 patients with GC/MRS from a Lyme borreliosis endemic area (median duration of disease, 8 months [range, 3-348 months]). *Borrelia burgdorferi*-specific DNA could not be amplified by polymerase chain reaction in any of the 12 patients. One (13%) of 8 patients tested had a serum *B burgdorferi* IgG response on enzyme-linked immunosorbent assay, and 2 patients (25%) had an IgM response, but immunoblot testing yielded negative results in all 8 patients.

Conclusion: The results of the present study do not indicate that *B burgdorferi* has an etiologic role in GC/MRS.

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MELKERSSON-Rosenthal syndrome (MRS) is a triad of chronic orofacial swelling predominantly involving the lips, relapsing peripheral facial nerve paralysis, and a furrowed dorsum of the tongue (lingua plicata).^{1,2} Monosymptomatic or oligosymptomatic forms in which only 1 or any 2 features of the triad are present are common.³ The most frequent monosymptomatic form is Miescher granulomatous cheilitis (GC),^{1,3,4} which is defined as painless chronic isolated enlargement of one or both lips due to granulomatous inflammation with a recurrent to gradually persistent course. Histopathologically, GC is characterized mainly by small, noncaseating epithelioid cell granulomas, sometimes with multinucleated giant cells of the Langhans type, which are sparsely scattered throughout the edematous connective tissue. Surrounding perivascular and interstitial infiltrates of lymphocytes, plasma cells, and histiocytes are also seen.^{3,5} Many forms of symptomatic treatment, including systemic or intralésional corticosteroids, have been used for

GC/MRS, but all have yielded only limited success.

The etiology of GC/MRS is unclear, although many theories have been advanced that suggest an allergic, infectious, autoimmune, or genetic cause.^{1,6-9}

Lyme borreliosis (LB) is a multisystemic infectious disease that is caused by the arthropod-borne spirochete *Borrelia burgdorferi sensu lato*.¹⁰ The spectrum of neurologic manifestations of LB includes various cranial nerve neuropathies, of which facial nerve paralysis is the most common.¹¹

For editorial comment see page 1557

An etiologic role of *B burgdorferi* has been suggested by some authors in various granulomatous diseases. *Borrelia burgdorferi* has been detected in lesions of GC and sarcoidosis by dark-field microscopy and culture.¹² Recently, *B burgdorferi*-specific DNA has been found in urine samples from 5 (38%) of 13 patients with granuloma annulare by polymerase chain reaction (PCR).¹³ A positive antibody re-

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PATIENTS AND METHODS

A retrospective case series involving 12 consecutive patients with GC (6 men and 6 women; age range, 33-77 years; median age, 45 years) seen at the Department of Dermatology, Karl-Franzens University School of Medicine, Graz, Austria, between 1990 and 1999 was examined in the present study (**Table**). The diagnosis was based on clinical and histopathologic criteria. Recurrent enlargement of the lip(s), which eventually persisted in cases with longer disease duration, had been present in all 12 patients over a period of 3 to 348 months (median, 8 months). Three patients (Nos. 8, 9, and 11) had a history of facial nerve paralysis 20, 28, and 18 years, respectively, prior to presentation. Four patients (Nos. 6, 8, 9, and 11) had been suffering from lingua plicata. One patient (No. 3) had an erythema migrans lesion on his right thigh 6 months before presentation, at which time GC had already been present. All patients resided in areas endemic for LB in Austria and had suffered multiple arthropod bites before the onset of disease. In all patients, various therapies were used before sampling, including intralesional (triamcinolone, 5-10 mg per injection) or systemic (prednisolone, 25-50 mg/d) corticosteroids, but showed no permanent effect. Two patients (Nos. 3 and 9) had received oral penicillin: patient 3 received a 2-week cycle of penicillin V sodium (1.5 million IU 3 times a day) because of his erythema migrans 6 months before the biopsy specimen was obtained, and patient 9 received 6 courses of penicillin V sodium between 10 and 2 years before the biopsy specimen was obtained, but both patients responded only temporarily to this therapy.

Punch biopsy samples from affected lip tissue, which were fixed in formalin and embedded in paraffin, were available from all 12 patients. These specimens were histopathologically examined and analyzed by PCR for the presence

of *B burgdorferi*-specific DNA as described.¹⁶ Briefly, after deparaffinization with xylene, samples were digested using proteinase K overnight. Subsequently, DNA was extracted by phenol-chloroform, precipitated with ethanol, and resuspended in distilled and autoclaved water. An aliquot of the DNA solution was used as a template for a nested PCR amplification, in which the first primer pair amplifies a 171-base pair fragment, and the second primer pair a 92-base pair internal fragment. The primer pairs anneal specifically to *B burgdorferi*-specific target DNA sequences described by Rosa et al.¹⁷ Amplification products were analyzed by agarose gel electrophoresis and visualized by UV light after ethidium bromide staining. Cultured strains of *B burgdorferi* and formalin-fixed, paraffin-embedded biopsy specimens from 5 erythema migrans lesions in which PCR amplification of *B burgdorferi*-specific DNA was successful in a former study were analyzed as positive control samples. Specimens obtained from 5 psoriatic skin lesions were used as negative control samples. All controls yielded the expected positive or negative results; 4 (80%) of the 5 erythema migrans samples tested positive by PCR. The IgG and IgM antibody responses to *B burgdorferi* were determined in serum samples drawn the same day as the skin biopsy specimens were obtained in 8 of the 12 patients by standardized enzyme-linked immunosorbent assay (ELISA) (Lyme Borreliosis ELISA Kit; Dako Corp, Glostrup, Denmark) as well as by immunoblot (*Borrelia* Western Blot IgG, IgM; Gull Laboratories, Bad Homburg, Germany) tests. On presentation, all patients underwent otorhinolaryngologic, dental, ophthalmologic, and neurologic examinations, and chest radiography was performed. Laboratory investigations included a complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, liver and renal function tests, serum electrolytes, creatine phosphokinase, antistreptolysin titer, and urinalysis.

sponse to *B burgdorferi* has been recognized in patients with sarcoidosis in significantly higher percentages than the respective seroprevalence rate,^{12,14} a finding that could not be confirmed by other authors.¹⁵

In the present study, molecular and serologic examinations were performed in 12 patients with GC/MRS to investigate a possible association with *B burgdorferi* infection.

RESULTS

On clinical examination, all 12 patients had nontender, soft to firm swelling of one or both lips, and 4 patients (Nos. 6, 8, 9, and 11) had a furrowed tongue (**Figure 1**). Facial paralysis was not present in any of the 12 patients. There was no evidence of skin, nervous system, joint, or cardiac manifestations of LB in any of the patients.

Histopathologic examination of the biopsy specimens from affected lip tissue revealed a moderate to dense, mainly superficial, but also deep perivascular and interstitial infiltrate composed of lymphocytes, histiocytes, and plasma cells in all patients. Noncaseating epithelioid cell granulomas with giant cells mainly located in the lower part of the lamina propria were observed in 8 patients (**Figure 2**). Edema was present in the upper part of the

lamina propria in 6 cases. No foreign bodies were seen when the sections were examined under polarized light.

Borrelia burgdorferi-specific gene segments could not be amplified by PCR in any of the lesional tissue biopsy specimens from the 12 patients.

One (13%) of 8 patients tested had a positive serum ELISA *B burgdorferi* IgG antibody result, and 2 patients (25%) had an IgM response (**Table**). Five patients had no serologic evidence of borreliosis. Immunoblot testing yielded negative results in all 8 patients.

Ear, nose, and throat examination, including computed tomography of the paranasal sinuses, revealed sinusitis in 4 patients. All sinuses were affected in patient 1, ethmoidal and maxillary sinuses in patient 2, and ethmoidal sinuses in patients 8 and 11. Sinusitis has been chronic and asymptomatic in all 4 patients. Generalized periodontitis was present in 4 patients (Nos. 1, 2, 7, and 10), but there were no signs of an infectious focus of the oral cavity in any of the patients. The findings of ophthalmologic and neurologic examinations were normal in all patients. Laboratory examination did not reveal any evidence of acute inflammatory or infectious foci.

In all patients, systemic and/or intralesional corticosteroid treatment (in combination with clofazimine in patient 9) was repeatedly administered after biopsy speci-

Clinical and Serologic Data on 12 Patients With Granulomatous Cheilitis and Their Treatment*

Patient No./Sex/Age, y	Duration of Disease, mo	<i>Borrelia burgdorferi</i> Serum ELISA IgG/IgM Antibodies	Antibiotic Therapy	Other Therapy
1/M/52	3	-/-	None	C/il
2/F/77	3	ND	Penicillin V sodium	C/s
3/M/43	14	+/-	Penicillin G sodium, minocycline hydrochloride	C/s
4/F/45	7	-/+	Penicillin G sodium	C/s
5/M/65	5	ND	Penicillin V sodium	C/s
6/F/33	3	-/-	Penicillin V sodium	C/s
7/M/45	9	-/+	Penicillin G sodium	C/s, C/il
8/F/36	240	-/-	None	C/s, C/il
9/M/46	348	ND	Penicillin G sodium	C/s, clofazimine
10/M/42	3	-/-	None	C/il
11/F/45	120	-/-	Penicillin G sodium, amoxicillin-clavulanate potassium	C/s
12/F/62	10	ND	None	C/s

*ELISA indicates enzyme-linked immunosorbent assay; -/-, negative for *B burgdorferi* IgG and IgM antibodies; +/-, positive for *B burgdorferi* IgG antibodies; -/+, positive for *B burgdorferi* IgM antibodies; ND, not done; C/il, intralesional corticosteroid injections (triamcinolone, 5-10 mg per injection); C/s, systemic corticosteroid therapy (prednisolone, 25-50 mg/d).

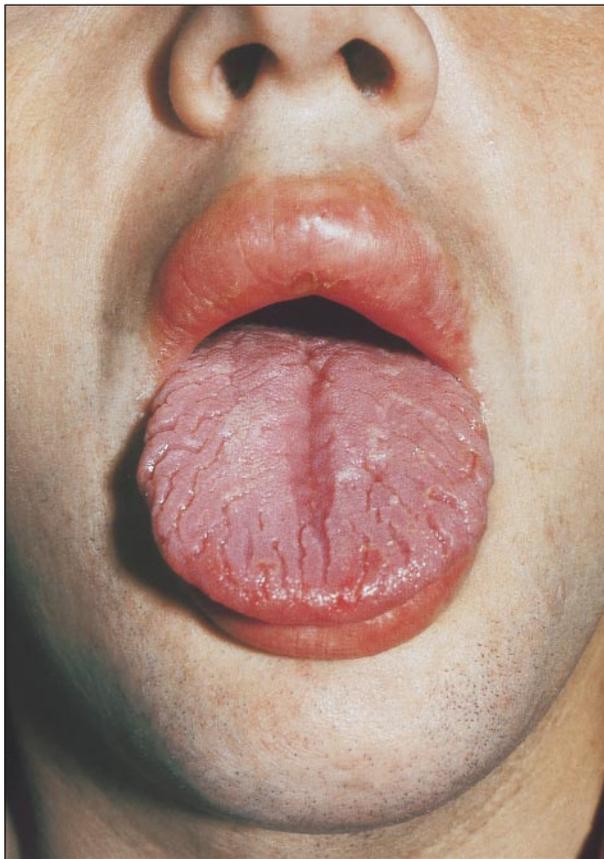


Figure 1. Patient 8. Diffuse swelling of the upper lip and furrowed tongue.

mens and serum samples were obtained at the first visit (Table). Eight of the 12 patients also received oral or intravenous penicillin therapy for at least 2 weeks. Patient 3 received an additional 2-week cycle of minocycline, and patient 11 received an additional 10-day cycle of amoxicillin. All patients showed only incomplete and transient improvement after corticosteroid as well as antibiotic treatment.

COMMENT

The cause of GC/MRS is still unknown. Among the numerous postulated theories are genetic factors,^{1,8} chronic infectious odontogenic foci,³ autoimmune mechanisms,¹ allergic reactions, including hypersensitivity to food additives,^{6,7} and local disturbances of the autonomic nervous system.⁹ A possible relationship with sarcoidosis^{5,18} or Crohn disease^{5,19} has been described, but GC/MRS is now believed to represent a separate and distinct entity.^{1,20}

It has also been suggested that GC/MRS may represent a type of infection; it has been ruled out, however, that *Toxoplasma gondii*, *Treponema pallidum*, mycobacteria, or herpes simplex virus are involved in the pathogenesis of GC/MRS.⁹ Another microbial agent, *B burgdorferi*, may be causative for this disease for several reasons. The spirochete has been detected directly in lesions of GC.¹² *Borrelia burgdorferi* infection also has been proved by direct methods (eg, culture and PCR of urine specimens) and/or serologic testing in other granulomatous diseases, including sarcoidosis and granuloma annulare.¹²⁻¹⁴ In areas that are endemic for LB, *B burgdorferi* is the leading cause of peripheral facial paralysis,²¹ which is not only a very important feature of Lyme neuroborreliosis but also a major sign of the triad of MRS. Other cranial nerves (eg, optic, oculomotor, and trigeminal) may be involved in Lyme neuroborreliosis¹¹ as well as in MRS.⁴ Plasma cells are a characteristic part of the uniform cell response in inflammatory infiltrates of LB lesions.²² Plasma cells may also be observed in small numbers scattered throughout the lamina propria of the normal oral mucosa, but the regular appearance of that cell type in higher numbers and in deeper parts of the lamina propria in GC may be indicative of an infection with *B burgdorferi*.

In the present study, etiologic factors could not be identified in any of the 12 patients with typical GC. In particular, no indications were found for (recurrent) facial erysipelas or for the coexistence of Crohn disease or

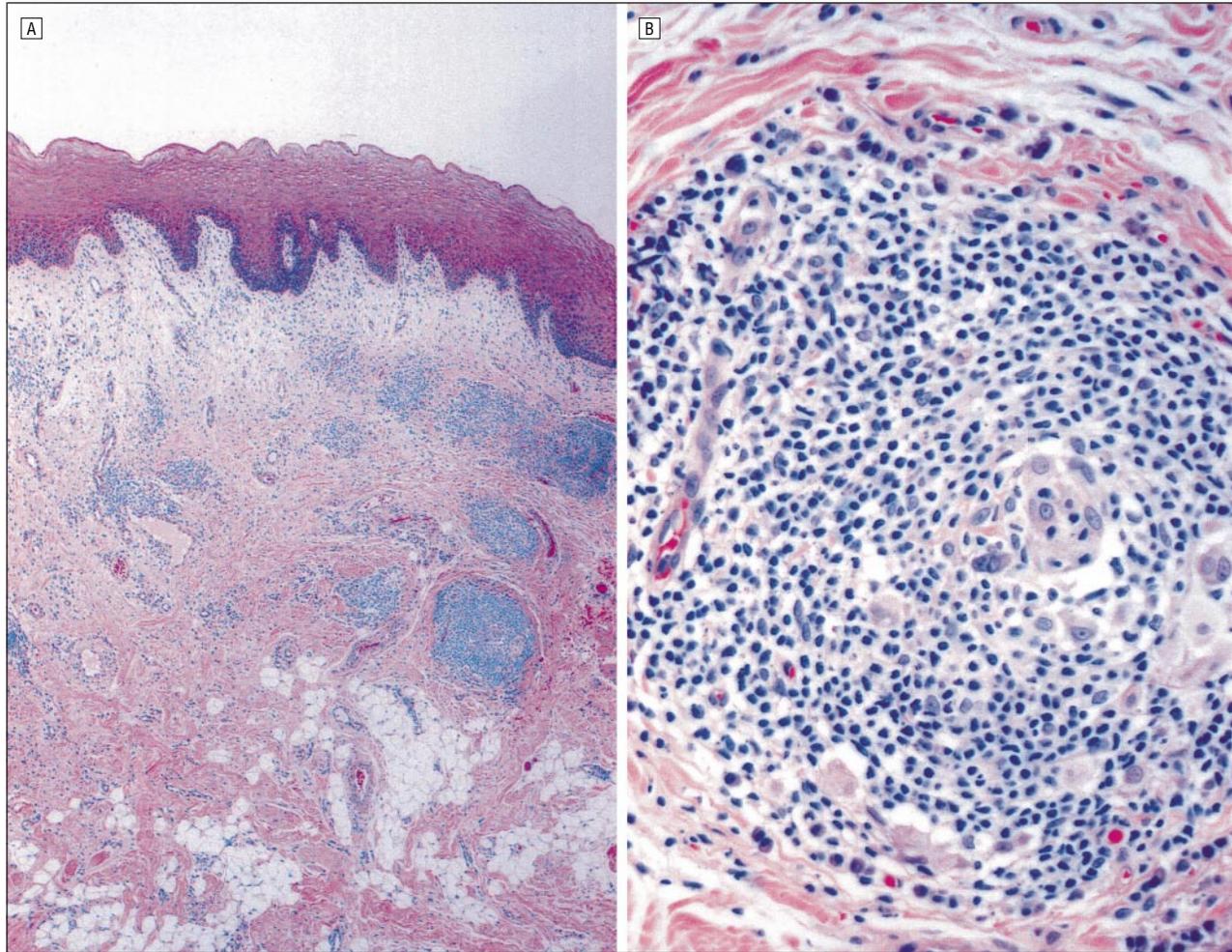


Figure 2. Patient 3. Histopathologic findings in a lip specimen. A, Superficial and deep perivascular mononuclear infiltrate with a noncaseating granuloma in the submucosal connective tissue (hematoxylin-eosin, original magnification, $\times 50$). B, Noncaseating epithelioid cell granuloma with several plasma cells in the periphery (hematoxylin-eosin, original magnification, $\times 250$).

sarcoidosis. There was no clear evidence of LB in the history or on presentation in any of the patients.

Borrelia burgdorferi-specific DNA could not be amplified in any of the biopsy specimens from the 12 patients. The results of PCR may have been negative because of certain technical issues that pertain to the analysis of formalin-fixed, paraffin-embedded tissue. However, we used a nested PCR procedure with primers for the amplification of relatively short *B burgdorferi*-specific gene segments that has been demonstrated to be a very sensitive method, even when applied to formalin-fixed, paraffin-embedded tissue.¹⁶ Moreover, the standard control experiments that we used in our protocol, including the examination of formalin-fixed, paraffin-embedded erythema migrans specimens, preclude false-negative results. Although the primers that we used are reactive with a wide variety of *B burgdorferi* strains,^{16,17} the possibility that GC may be caused by a *B burgdorferi* subtype that is not detected by means of our PCR method cannot be ruled out. Antibiotic treatment prior to the time of the punch biopsy may be responsible for a negative PCR result. However, only 2 of our 12 patients were treated with short courses of penicillin up to 6 months and 2 years, respectively, before sampling.

One (13%) of 8 patients tested (No. 3) had an IgG ELISA antibody response to *B burgdorferi*, and 2 patients (25%) had an IgM ELISA antibody response, but the positive ELISA results could not be confirmed by immunoblot testing. The constellation of a positive IgG ELISA result but a negative immunoblot result in patient 3 is compatible with his recent erythema migrans. Detection of IgM antibodies to *B burgdorferi* in the other 2 patients may point to active or recent LB. However, the diagnostic specificity of IgM assays is often affected by polyclonal B-cell activation,²³ and positive IgM titers resulting from this may persist for a long time. Furthermore, all patients have resided in areas endemic for LB in Austria and had suffered multiple arthropod bites before the onset of disease. Studies in such areas, including the regions in which our patients have been living, have demonstrated the prevalence of *B burgdorferi* antibodies to range between 8% and 45%.^{24,25} Thus, the results of serologic testing in our series are not significantly different from the expected seroprevalence rate.

At some time after the punch biopsy, 8 of the 12 patients received at least 1 course of antibiotic treatment. We observed some improvement in all 8 patients dur-

ing or shortly after the antibiotic treatment, but eventually all patients had a relapse. There are few reports concerning antibiotic treatment of GC in the literature. Treatment with tetracyclines has been found to be ineffective³ or occasionally effective.^{26,27} Apparent effectivity of antibiotics, however, may result from their combination with prednisone²⁶ or from anti-inflammatory- and biologic response-modifying features rather than from an antimicrobial action.²⁸ Thus, the limited response to antibiotics, which are appropriate for the treatment of LB, in our series, as well as in the literature, is not a strong indicator of an infectious origin (eg, *B burgdorferi*) of GC/MRS.

In summary, the results of our study on a retrospective case series of 12 patients imply that *B burgdorferi* is not involved in the pathogenesis of GC/MRS. Additional and larger prospective studies, including culture and molecular analyses of frozen tissue, are needed to further assess a possible association between GC/MRS and *B burgdorferi* infection.

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