FACIAL GRANULOMA ANNULARE ASSOCIATED WITH BORRELIA BURGDORFERI INFECTION AND SEROPOSITIVITY FOR P25, P30 AND P31 PROTEINS

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ABSTRACT

Lyme disease is a multisystemic infectious disease caused by Borrelia burgdorferi spirochetes, transmitted by ticks. Beside the typical clinical lesions of the disease, some other skin diseases associated with Borrelia burgdorferi infection have also been reported in recent years. In the article, we present a 39-year-old woman with facial granuloma annulare who was bitten by a tick 20 months ago. Beside the borrelial antibodies, other clinical and laboratory findings of the patient were normal. Histopathology of the lesion was compatible with granuloma annulare. Borrelia IgM was negative and IgG was positive in the ELISA test of the peripheral blood. In the Western immunoblotting test, anti-borrelia IgG was positive for p25, p30 and p31 proteins. Based on the findings, the lesion was thought to be associated with Borrelia burgdorferi infection and the patient was treated with doxycycline. The lesion completely disappeared 7 weeks after the initiation of the treatment. Our case was presented because no case of granuloma annulare associated with borrelia proteins of p25, p30, p31 have been reported in literature. Additionally, a possible cross-reaction of T-cell type which might have induced the lesion was discussed through the concepts of "molecular mimicry".

Keywords: *Borellia burgdorferi, granuloma annulare, Lyme disease, molecular mimicry.* ***Nobel Med 2015; 11(3): 84-88***

BORRELİA BURGDORFERİ İNFEKSİYONU İLE İLİŞKİLİ FASİYAL GRANÜLOMA ANÜLARE VE P25, P30 VE P31 PROTEİNLERİ İÇİN SEROPOZİTİFLİK

ÖZET

Lyme hastalığı, kene ile taşınan Borrelia burgdorferi spiroketlerinin neden olduğu multisistemik bir infeksiyon hastalığıdır. Hastalığın tipik klinik lezyonları dışında, son yıllarda bu spiroket infeksiyonu ile ilişkilendirilen başka bazı deri hastalıkları da bildirilmiştir. Biz bu makalede, 20 ay önce bir kene tarafından ısırılmış fasiyal granüloma anülareli 39 yaşındaki bir kadın hastayı sunuyoruz. Hastanın borelyal antikorları dışındaki diğer klinik ve laboratuar bulguları normaldi. Lezyonun histopatolojisi granuloma anülare ile uyumluydu. Periferik kan ELISA testlerinde, anti-borelya IgM negatif ve anti-borelya IgG pozitifti. Western blotting testinde ise borelyanın p25, p30 ve p31 proteinlerine karşı gelişmiş IgG antikor pozitiflikleri saptandı. Bu bulgular ışığında lezyonun bu spiroket infeksiyonu ile ilişkili olduğu düşünülerek hasta doksisiklinle tedavi edildi. Lezyon tedaviye başlandıktan 7 hafta sonra tamamen geriledi. Hastamız, Borrelia'nın p25, p30 ve p31 proteinleri ile ilişkilendirilmiş herhangi bir granüloma anülare olgusuna literatürde rastlanmaması nedeniyle sunulmuştur. Ayrıca, bu lezyonun oluşumunu indükleyebilecek T-hücre kaynaklı olası bir çapraz reaksiyon, "moleküler benzerlik" kavramı üzerinden irdelenmiştir.

Anahtar kelimeler: Borrelia burgdorferi, granüloma anülare, Lyme hastalığı, moleküler benzerlik **Nobel Med 2015; 11(3): 84-88**

INTRODUCTION

Lyme disease (LD) is a multysystemic infectious disease caused by Borrelia burgdorferi (Bb) spirochetes, as a result of a tick bite. The three characteristic cutaneous manifestations are erythema migrans (EM), lymphadenosis benigna cutis, and acrodermatitis chronica atrophicans.[1] Besides the classical manifestations of cutaneous borreliosis, evidence is growing that at least in part, other skin manifestations, especially morphea, lichen sclerosus and cases of cutaneous B-cell lymphoma are causally related to infections with borrelia.[2]

There are also single reports of other skin manifestations associated with borrelia infections like cutaneous sarcoidosis, necrobiosis lipoidica, necrobiotic xanthogranuloma, systemic sclerosis, eosinophilic fasciitis, atrophoderma of Pasini and Pierini, pseudolymphoma, septal panniculitis resembling erythema nodosum, progressive facial hemiatrophy of Parry-Romberg, sclerodermatous porphyria cutanea tarda, interstitial granulomatous dermatitis and granuloma annulare (GA).[2-4]

CASE

A 39-year-old Caucasian woman was admitted to the hospital with persistant and spreading lesion for 9 months. According to the history, the patient was bitten by a tick 20 month ago. One month after she was bitten, an itchy, annular and erythematous patch emerged at the bite site and it recovered 2 months later. The patient had no therapy for the lesions and she had been in good health until 9 months ago. Then, the patient noticed a papular lesion on the right cheek. The lesion gradually enlarged over a 9 month period until her admittance to the hospital. She was given oral and topical antihistaminics and topical steroids for the lesions which did not heal. In the dermatological examination, a red-colored, annular lesion (38 mm in size) which was composed of small papules, on the right zigomatic region was seen. The middle of the annular lesion was pale and slightly atrophic (Figure 1a).

Other dermatological and systemic examinations and laboratory tests (including peripheral blood smear, blood glucose values, HbA1c, rheumatoid factor, antinuclear antibody, anti-ds DNA, tumor markers VDRL, TPHA and anti-HIV antibodies) were negative or within the normal limits. In the enzyme-linked immunosorbent assay (ELISA) tests of the peripheral blood, anti-borrelia IgM was negative (4 RU/ml) and IgG was positive (21 RU/ml) [cut-off value: 16 RU/mL, Evaluation of ELISA tests: <16 RU/mL= negative, 16-22 RU/mL=borderline positive, ≥22 RU/mL=positive, with EUROIMMUN anti-borrelia ELISA test, Medizinische Labordiagnostika AG, Lübeck, Germany].

In the Western immunoblotting (WB) tests, anti-borrelia IgM was negative (Figure 2a), and anti-borrelia IgG was strong positive against the p30, p31(OspA) proteins and weak positive against the p25 protein. Immune responses to other antigens of Bb [VLsE, p17, p19, p21, p39] were negative (Figure 2b) [Evaluation of WB tests: 2 band positivity=strong positive, 1 band positivity=positive, band negativity=negative, with EUROIMMUN anti-borrelia EUROLINE test, Medizinische Labordiagnostika AG, Lübeck, Germany]. In the histopathological examination of a 2-mm punch biopsy specimen, there was a palisade-patterned granuloma with a central zone of necrobiotic collagen surrounded by a palisade of histiocytes and some lymphocytes which settled between and around the collagen bundles in the superficial dermis (Figure 3). Based on these findings, the lesion was diagnosed as GA associated with the late-stage borrelia infection. The patient was treated with oral doxycycline for 4 weeks (200 mg/day) and monitored weekly. Additional systemic or topical treatment was not given to the patient. No adverse effects were observed during the treatment and the lesion gradually decreased in three weeks. The lesion disappeared completely seven weeks after the initiation of the treatment leaving a post inflammatory hyperpigmentation (Figure 1b). The patient is still under follow-up. During a 11 month follow-up period the facial lesion did not recur.

DISCUSSION

GA, is an idiopathic and benign granulomatous disorder with classical features including single or grouped papules which slowly enlarge into an annular shape. The subsets of the disease are localized, generalized, perforating, subcutaneous and erythematous types.[5] The lesion of our patient was in a localized form. Despite the fact that the etiopathogenesis of GA is unknown, it has been thought that it is a reaction pattern to many different triggering factors.[6] It has been reported in patients with diabetes mellitus, malignancies, thyroid diseases, hepatitis B and C virus infections, BCG vaccination and herpes virus infection.[5,7]

The relation between GA and Bb was firstly reported by Strle et al. in 1991.The authors demonstrated the presence of borrelia in 85% of localized GA cases by using immunohistochemistry and focus-floating microscopy. Nevertheless, PCR tests for borrelia were positive in only 5.9% of localized GA lesions.[8] In 1999 Aberer et al. showed that Bb flagelin gene sequences could be detected by PCR in the urine of 61% of patients with GA.[9] In 2008, Ziemer et al. reported that

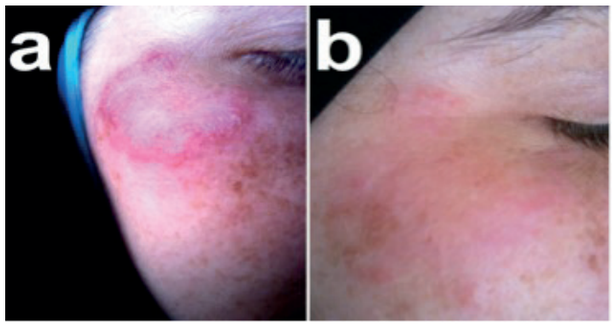

Figure 1. Clinical views of the lesion: before the treatment (a), and 7 weeks after the doxycycline therapy (b).

borrelia antibodies were detected in 127 of 157 biopsies of localized GA, and they reported that borrelia might have been a part of the aetiology.[10]

Two years later, Zollinger et al. reported that Bb DNA was detected in only one of the 48 GA biopsies by PCR, and they stated that the association between the GA and Bb could not be argued.[3] Chandra et al. showed significantly higher antibodies against Bb proteins p28, p30, p31, p34 by WB in 54 serum specimens with borrelia-seropositive post-LD patients compared to post- LD healthy subjects. Aguero-Rosenfelt et al. stated that some Bb proteins such as flagellin (41 kDa), FLaA (37 kDa), OpsA (31 kDa), OpsB (34 kDa), OpsC (21 to 25 kDa), BmpA (39 kDa), VLsE were highly immunogenic, and certain flagellin epitopes were also cross-reactive with antigens found in mammalian tissues such as neural tissues, synovium, and myocardial muscle.[11] Dai et al. indicated that the flagellin (41 kDa) cross-reacts with human axons and the protein can play a role in LD through the molecular mimicry.[12] One of the highly immunogenic proteins of Bb is p83 which is similar to eukaryotic cell structures. Rössler et al. speculated that it might be involved in the mechanisms of LD by mimicking these structures.[13]

Tas et al. reported a case with generalized GA who resisted antihistamines and topical corticosteroids. In WB tests of their patient, anti-borrelia IgM was positive against p83, and IgG was negative. The patient was treated with doxycycline as it was considered as early-stage of LD.[14] In the course of having LD, a specific stage for GA has not yet been defined. Strle F et al. stated that the emergence of the lesions of GA can begin within two months after the tick bite, and can last up to one year.[8]

The annular plaque of our patient appeared 11 months after the tick bite, and it was compatible with the literature. GA has also been observed in individuals with cell-mediated immunity deficiencies such as sarcoidosis.[7] Due to the presence of activated T-cells in the lymphocytic infiltrate of GA, it has been suggested that a T-cell-mediated delayed-type hypersensitivity response is the cause of the GA.[7,15] Fayyazi et al. have suggested that in GA, IFN-γ secreting Th-1 lymphocytes may cause a delayed-type hypersensitivity reaction. In this reaction, macrophages are differentiated into effector T-cells which express TNF-α and matrix metalloproteinases.[15]

In a different aspect, molecular mimicry is one of the mechanisms by which infectious agents trigger an immune response against the host antigens. When a susceptible host which is infected with a microorganism that has antigens immunologically similar to the host antigens but has different authigenic epitopes to induce an immune response when presented to T cells, the host may fail to tolerate its own antigens. Furthermore, a pathogen-specific immune response that cross-reacts with host structures may develope, and it may cause tissue damage and disease.[16,17] Autoreactivity is based on antigenic cross-reactivity between similar epitopes of borrelia and the human host, especially situated on the heat shock proteins (Hsp). Many Hsp of Bb such as p60, p66, p43, p72, p24, p35, p28 have been identified.[13,18] Some diseases have been reported which might be associated with the molecular mimicry, such as insulin-dependent diabetes, LD and syphilis.[11,17]

Tchernev and et al. have stated that an autoimmune etiology of sarcoidosis could possibly occur through a process of molecular mimicry between infectious or other environmental antigens and host antigens.[19] Moreover, it has been stated that the lyme arthritis might be the result of a persistent Bb infection in which borrelial DNA and antigens were retained in the patient, and these DNA products might be responsible for induction of a cross-mediated autoimmunity due to a T-cell-receptor epitope mimicry.[20]

On the other hand, cultivation of borrelia from a patient's skin or blood is the gold standard but it is expensive and lacks sensitivity. Detection of spirochetal DNA by PCR has higher sensitivity, but PCR for detection of Bb has not yet been standardized.[21,22] Detection of antibodies to Bb is the most practical and common approach.[21] The two-tier test approach in which a positive or indeterminate result of an ELISA test followed by a more specific WB assay for verification, still provides the physician with a reasonably accurate and reliable assessment of anti-Bb antibodies.[21,22] Anti-borrelia IgM rises 2-4 weeks after the tick bite and may persist at high levels while anti-borrelia IgG might not rise for many years.[23,24]

Moreover, both IgM and IgG may persist for many years after successful treatment of LD. In late LD course, usually, high antibody concentrations and

numerous immunoreactive bands in IgG immunoblots are detected.[20] It has been postulated that usually antibodies against some immunodominant borrelia proteins such as p41, OspC (p25), p35, p37, p45 might be detected at the early-stage of LD whereas some others such as p31(OspA), p34(OspB), p28 and 30 might be detected at the late-stage of LD.[13] Aguere et al. have stated that the antibodies against p39 were detected at the convalescence phases.[11]

In our case, we did not find another reason that may be responsible for the etiology of the disease except a strong IgG positivity against the two borrelia antigens and a weak IgG positivity against another protein. When the time elapsed after the tick bite (20 months), the time of appereance of the EM (1 month after the biting), the subtypes and strengths of positive antibody bands of IgG (weak positivity of the OspC (p25) and strong positivity of both p30 and p31), and negativity of the IgM antibodies were taken into account, the stage of our patient was more compatible with late-stage of LB.

Our findings supported to the findings of Aguere et al.'s. Based on this information, we thought that an antigenic mimicry may be responsible for the clinical picture of GA. In this case, a crossed-mediated reaction due to the T-cell type immune response might have been developed between the host proteins and borrelia antigens, and the proteins (p25, 30 and 31) of Bb might have been etiologically responsible for acting as triggers of the pathogenicity as Hsp of spirochaetes. We also thought that GA of our patient might be one of the presentations of the late-stage of LD that developed 20 months later.

In the histopathology of GA, a palisade-patterned granuloma with a central zone of necrobiotic collagen surrounded by a palisade of histiocytes and some lymphocytes are shown, as in our patient. Differential diagnoses include tinea, annular lichen planus, erythema annulare centrifugum, EM, erythema multiforme, tuberculides, sarcoidosis, tertiary syphilis and mycosis fungoides.[25,26] Due to the absence of foamy histiocytes, interface changes, caseification, atypical lymphocytes, neutrophils, and typical histopathology of our lesion, those diagnoses were excluded.

LD should be treated in all stages of the disease.[20] Doxycycline is the mainstay of therapy of cutaneous manifestations of borrelia infections. In the late skin manifestations of the LD, it is recommended that the duration of the treatment should be at least four weeks.[1,20] Due to the strong positivity of the late-stage antibodies, we treated our patient with doxycycline for four weeks. Our case was reported to draw attention to the possible relation of pathogenesis of GA with the borrelia antigens according to the concepts of the molecular mimicry.

Figure 2. Negativity of the anti-borrelia IgM in the WB test (a). Picture of immunoblots for this patient showing intensity diagram (top) band intensities (middle) and ruler of the band profile of the reactive bands (bottom) (b).

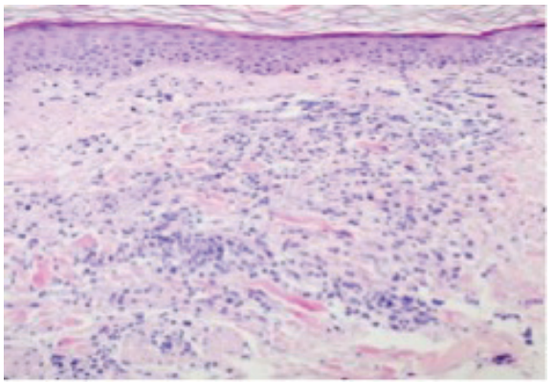

Figure 3. Histopathology of the granuloma annulare (A central zone of necrobiotic collagen bundles surrounded by a palisade of histiocytes and some lymphocytes in the superficial dermis) HE x10

CONCLUSION

Beside the typical clinical lesions of LD, some other skin diseases associated with Bb infection have also been reported in recent years including GA. The exact etiopathogenesis of GA is still unknown. We presented a patient with GA who has a history of tick bite. In the etiopathological investigation of our patient, we detected some borrelia proteins such as p25, 30 and 31 as immunoreactive bands in the WB tests. The lesion disappeared completely in seven weeks with doxycycline therapy.

Our case was presented because no case of GA associated with borrelia proteins of p25, p30, p31 have been reported in literature. We thought that an antigenic mimicry may be responsible for the pathogenesis of the GA, and these proteins of Bb might have been etiologically responsible for acting as triggers of pathogenesis as Hsp's of the spirochaete. We believe that more detailed investigations of future cases with similar characteristics (have a history of a tick bite and immunopositivity for the Bb proteins) will enlighten the mechanism of pathogeneses of LD and GA associated with Bb proteins.

* The authors declare that there are no conflicts of interest.

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