Granuloma annulare displaying pseudorosettes in Borelia infection

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ABSTRACT

Aims: In 2003, pseudorosettes were described as highly suspicious of infection by *Borrelia burgdorferi* sensu lato in the appropriate clinical context. Nevertheless, such a pattern has been described in the literature in other non-infectious conditions. On the other hand, granuloma annulare (GA) has been recently closely associated with infection by *Borrelia*. We investigated how frequently pseudorosettes can be detected in common GA cases confirmed for *Borrelia* by PCR.

Methods and results: We studied 13 biopsies of non-interstitial GA and 2 biopsies of interstitial GA from patients without clinical suspicion of borrelial infection. We also performed immunohistochemical studies in all the biopsies, using the CD-68 antibody. Molecular studies with PCR were performed with β-globin PCR (human DNA). Borrelial DNA was confirmed by amplifying the *OspA* gene and intergenic *rrf-rrl* region. We found histiocytic pseudorosettes in 13 biopsies (86.66%). Human DNA was successfully amplified from 8 of 13 paraffin-embedded skin samples. From these we amplified borrelial DNA in 5 of 8 samples. Out of the 8 cases in which human DNA was amplified, a correlation between pseudorosettes and the molecular tests (*Borrelia* DNA) was confirmed in 5 instances.

Conclusions: a) Pseudorosettes are not an unusual finding in common granuloma annulare; b) *Borrelia* is present in (most) cases of granuloma annulare; and c) Pseudorosettes seem to be a good morphological sign predictive of infection with *Borrelia* in granuloma annulare.

KEY WORDS

granuloma annulare, pseudorosettes, macrophages, CD68, PCR

Introduction

In 2003, a constellation of clinical and morphological signs were presented as a peculiar manifestation of *Borrelia burgdorferi* sensu lato infection (1). An interesting type of granuloma was described in some biopsies from patients affected with Lyme disease clinically mimicking morphea. It was described by the authors as a "histiocytic pseudorosette," with a "free-floating collagen bundle, entirely surrounded by histiocytes." A pseudorosette was usually found in the interstitial dermal inflammatory infiltrate of histiocytes in granuloma annulare (GA), in patients with peculiar cutaneous manifestations.

Although the authors admitted that the same histologic features may be seen in lesions that are negative for *Borrelia* in PCR, they also suggested that the reason for this might have been a scant number of microorganisms. It should be mentioned that histiocytic pseudorosettes had previously been interpreted as a drug reaction that resolved after discontinuation of the drug in question (2).

In 2008, a study on evidence of *Borrelia* infection was published (3). In it, the authors demonstrated the presence of *Borrelia* in 85% of cases of localized GA by using immunohistochemistry and focus-floating microscopy. Nevertheless, PCR tests for *Borrelia* were positive in only 5.9% of localized GA lesions.

Additional techniques for detecting *Borrelia* are not always available to the general pathologist. Based on this, we investigated the morphological sign of "pseudorosettes" in cases of localized GA, and the molecular evidence of *Borrelia burgdorferi* infection by PCR.

Materials and method

We examined 13 biopsies of localized GA and two biopsies of interstitial GA. There was no clinical suspicion of Lyme disease in any of the cases, and the patients' only complaint was the presence of the GA lesions. The patients' ages and the locations of the lesions are presented in Table 1.

We looked for evidence of "histiocytic pseudorosettes" in routine hematoxylin-eosin stained slides. In order to prove that macrophages were involved in pseudorosettes, we performed immunohistochemical investigations in all the biopsies, using the CD-68 test (monoclonal mouse anti-human antibody, clone PG-M1, DAKO, and ready-to-use).

In order to perform the molecular study, we obtained two 4 mm sections from each paraffin-embedded block from 13 of the cases (in cases 4 and 5, the blocks were not found in the archives). The tissue was deparaffinized by two washes (15 min) with xylene that was removed by two washes (15 min) with 100% ethanol. DNA was extracted with a QIAamp® DNA MiniKit (Qiagen, Santa Clara, CA, USA) according to the manufacturer's instructions (4).

The extracted DNA was subjected to three different PCR procedures. We amplified human DNA with β-globin PCR. Borrelial DNA was confirmed by amplifying the *OspA* gene and intergenic *rrf-rrl* region. PCR reactions were carried out in separate rooms, and panels of positive and negative control samples were included in each experiment to avoid and monitor amplification and contamination. At least three runs were performed on each sample, in particular PCR (5).

The quality of each DNA sample was verified by amplifying the 268 bp fragment of the gene for human β-globin using PC04 and GH20 primers. Amplicons were visualized on a 3% agarose gel stained with ethidium bromide (6).

OspA *PCR*

The extracted DNA was subjected to nested PCR targeting of the OspA gene of *B. burgdorferi* sensu lato. The reaction was carried out for 30 cycles with the following conditions: 95 °C for 45 s, 50 °C for 45 s and 72 °C for 60 s. Each sample was transferred to a second reaction and amplified under the same conditions for another 30 cycles. Amplification products were analyzed on ethidium bromide-stained 1% agarose gel (7).

Nested PCR of intergenic rrf-rrl *region and RFLP analysis*

PCR amplification was performed using external primers SPA1 and SPA2 and internal primers P1 and P2 by one cycle of 3 min at 94 °C followed by 20 cycles (3 min at 93 °C, 2 min at 70 °C and 2 min at 72 °C), followed by 40 cycles (1 min at 93 °C, 2 min at 50 °C and 2 min at 72 °C) and followed by 7-min holding at 72 °C. PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis by digestion with 5U of *Mse*I restriction enzyme (Biolabs, New England), electrophoresed in a 16% acrylamide gel, and compared with the RFLP of a particular *B. burgdorferi* sensu lato species (8).

Results

We found pseudohistiocytic rosettes in 13 of the 15 biopsies (86.66%; Table 2). Figure 1 illustrates several examples of histiocytic pseudorosettes that were found in the biopsies, displaying the morphological criteria described by Moreno et al. (1). They were mainly located in the periphery of the granuloma, but in some biopsies they could be found in more central areas where the collagen fibers had a degenerated aspect; Figure 2.

CD68 was expressed by the granulomas cells in all the biopsies. Figure 3. Human DNA (β-globin) was successfully amplified from 8 of 13 paraffin-embedded skin samples.

In samples with successfully extracted DNA, we amplified borrelial DNA with nested PCR for *OspA* in 5 of 8 samples, whereas no positive sample was found by PCR of the intergenic *rrf-rrl* region; in other words, taking into account that human DNA was successfully amplified in 8 cases. Pseudorosettes were present in 6 cases and there was evidence of *Borrelia burgdorferi* in 4 cases, whereas PCR failed to demonstrate the presence of the microorganism in the other two cases. In contrast, in cases with no evidence of pseudorosettes (2 cases), *Borrelia* was demonstrated in one.

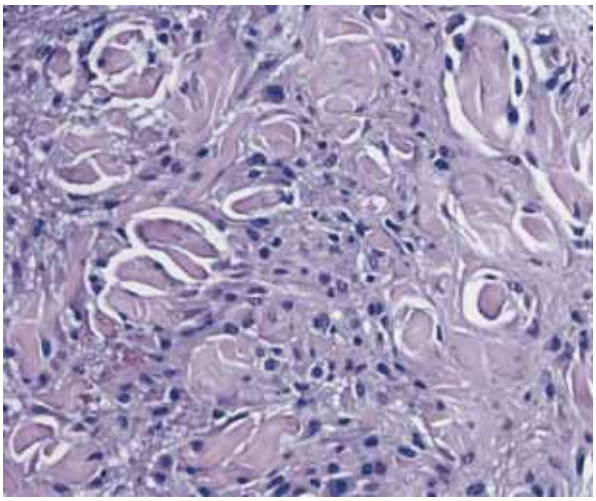

Figure 1. Histiocytic pseudorosettes found in our biopsies that met the morphological criteria described in reference number 1. There is a thick collagen bundle, entirely surrounded by histiocytes.

In total, there was correlation between pseudorosettes and the molecular tests (*Borrelia* DNA), in 5 of 8 cases.

Discussion

In 2003, Moreno et al. presented a type of granuloma that they considered to be closely associated with infection by *Borrelia*, which they called a "histiocytic pseudorosette" (1). All their patients presented with similar clinical manifestations, as well as similar histologies. These granulomas were found in a morphological background that looked like an interstitial GA.

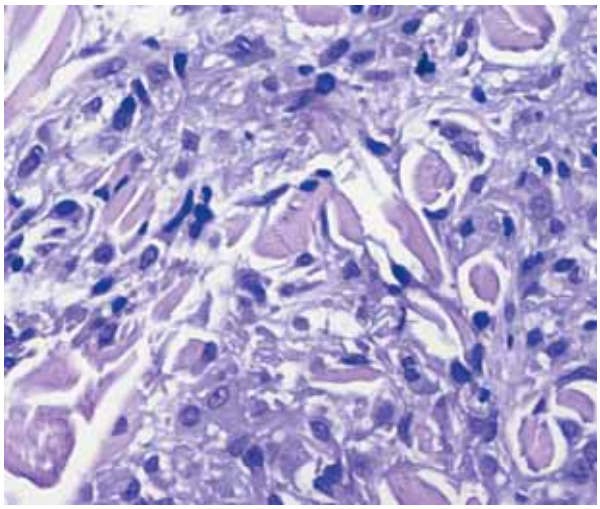

Figure 2. Most pseudorosettes were found at the periphery of the granuloma annulare, but some were found in more central areas, in which case they mixed with collagen fibers, presenting a degenerated appearance.

On the other hand, Ziemer et al. recently demonstrated *Borrelia* in a high percentage of localized GA (3). They used focus-floating microscopy (FFM), which they demonstrated to be more sensitive than PCR when looking for minuscule organisms. In fact, their PCR results in GA were rather poor (only one positive case out of 27).

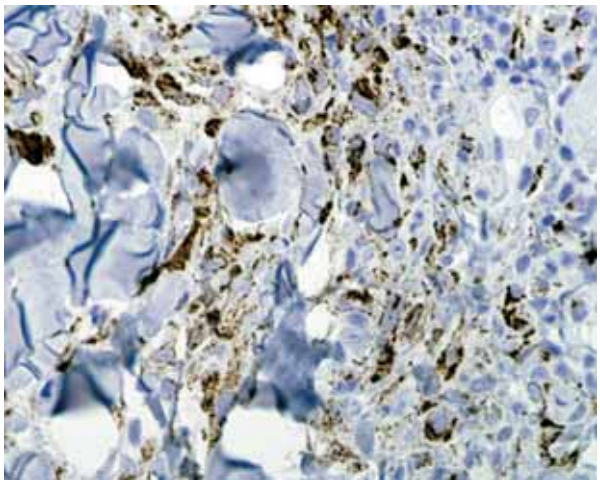

Figure 3. CD68 stain showing expression of the marker by the cells that surround the cracked collagen fibers.

We found pseudorosettes in a high percentage of our cases of GA. PCR demonstrated that most of the cases with pseudorosettes were positive for *Borrelia burgdorfer*, even taking into account the low sensitivity claimed for this technique. Our studies with β-globin-globin PCR, although different from the DNA extraction and amplification rates of 61.5%, are similar to the data reported in literature. The differences obtained with the nested PCR for *OspA* and intergenic *rrf-rrl* region may be explained by the possibility that borrelial DNA (as well as human DNA) was destroyed during the extraction process, and this affected the intergenic *rrf-rrl* DNA region more than the gene for *OspA*. Because there was no positive intergenic *rrf-rrl* PCR, we were not able to identify *Borrelia* species in the skin samples.

Moreno et al. admitted that similar morphological features can be found in other *Borrelia*-negative conditions. Even so, they insisted that in these conditions the possibility of a scant number of microorganisms should be considered. The results recently published by Ziemer et al. (3), as well as our own results, seem to point in that direction.

It should nevertheless be mentioned that similar pseudorosettes have previously been described in the literature in certain non-infectious, drug-associated transient conditions (2).

Many sclerosing cutaneous lesions have been suggested as possible forms of Lyme disease, such as morphea (9–15), systemic sclerosis, eosinophilic fasciitis (16, 17), lichen sclerosus et atrophicus (12), atrophoderma of Pasini and Pierini, pseudolymphoma, septal panniculitis resembling erythema nodosum (18), and progressive facial hemiatrophy of Parry-Roberg and sclerodermatous porphyria cutanea tarda (19). It could be argued that tests such as the ELISA assay or the immunofluorescent assays can be falsely positive or negative, and that cross-reactions with other infectious agents are possible (20, 21). The technique applied by Ziemer et al. clearly opens new ground for investigation in many of these conditions.

The relationship between GA and *Borrelia burgdorferi* is not a new topic in the literature (10). The isolation by culture of *Borrelia* from a GA in a patient with borderline serologic titters for *B Burgdorferi* sensu lato was reported (22). On top of that, flagelin gene sequences could be detected by PCR in the urine of 61% of patients with GA in one European series (23): this observation is close to the percentage in which we have found the pseudorosettes.

The open question is, obviously, whether *Borrelia* is the "only" cause of GA. If it is not, can the evidence of pseudorosettes be interpreted as equal to presence of the microorganisms? Our studies seem to favor that claim.

Also, it is worthy of note that the pseudorosettes were mainly located in the periphery of the granulomas. This location is the same as the one where Ziemer et al. found the highest concentrations of *Borrelia*, which may suggest a topographic relationship between the morphological sign (the pseudorosette) and the infectious cause.

Conclusions

A) Histiocytic pseudorosettes are a common finding in localized GA lesions. B) *Borrelia* DNA was amplified from most cases of granuloma annulare that were investigated. C) Pseudorosettes seem to be a good predictive sign of infection by *Borrelia* in GA.

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