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CONCISE COMMUNICATIONS

Absence of Kaposi's Sarcoma–Associated Herpesvirus DNA in Bacillary Angiomatosis-Peliosis Lesions

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Bartonella henselae **and** ***B. quintana*** **induce an unusual vascular proliferative tissue response known as bacillary angiomatosis (BA) and bacillary peliosis (BP) in some human hosts. The mechanisms of** ***Bartonella*****-associated vascular proliferation remain unclear. Although host factors probably play a role, microbial coinfection has not been ruled out. Because of the vascular proliferative characteristics noted in both Kaposi's sarcoma (KS) and BA and occasional colocalization of KS and BA, the possibility was explored that KS–associated herpesvirus (KSHV) might be associated with BA lesions. Tissues with BA and positive and negative control tissues were tested for the presence of KSHV DNA by a sensitive polymerase chain reaction assay. Only 1 of 10 BA tissues, a splenic biopsy, was positive in this assay; this tissue was from a patient with concomitant KS of the skin. Thus, KSHV is probably not involved in the vascular proliferative response seen in BA-BP.**

Vascular proliferation is characteristic of the local tissue response to several microbial pathogens, including *Bartonella bacilliformis, B. henselae,* and *B. quintana.* When immunocompromised persons are infected with the latter two species, bacillary angiomatosis (BA) and bacillary peliosis (BP) may develop [1, 2]. In contrast, immunocompetent persons rarely develop BA-BP [3]; they more typically develop granulomatous (cat-scratch disease) and bacteremic disease (trench fever) without angioproliferation during infection with *B. henselae* and *B. quintana,* respectively [4].

At least four hypotheses explain the variable host response to *B. henselae* and *B. quintana.* First, the state of immune competence determines whether host angioproliferative factors are expressed locally; second, only certain *Bartonella* strains with angiogenic potential circulate among immunocompromised persons; third, greater degrees of local *Bartonella* replication occur in the face of host immunosuppression, resulting in greater bacterial production of vascular proliferative factors; and fourth, BA and BP occur only in the presence of a coinfecting agent with angiogenic properties [5].

The ability of one pathogen to modify the host response to a coinfecting organism is an increasingly common theme [6]. Kaposi's sarcoma (KS)–associated herpesvirus (KSHV) is the putative cause of KS, a disease characterized by vascular proliferation [7]. Many of the persons at greatest risk for BA and BP are also infected with KSHV. The local host cellular responses to KSHV might create a permissive or favorable environment for the formation of BA. The striking colocalization of macroscopic KS and BA cutaneous lesions in several patients (see case report below) also stimulated our interest in investigating this hypothesis. The question was whether KSHV could be detected at the molecular level in lesions that appeared to be BA without KS at the macroscopic level. Therefore, we investigated the possible coassociation of *Bartonella* and KSHV within the vascular proliferative lesions of BA, using assays based upon the polymerase chain reaction (PCR).

Materials and Methods

Patients and tissue specimens. We studied 10 tissue specimens with pathology typical of either BA or BP (BA1–BA10) and 3 tissue specimens with pathology typical of KS (KS1–KS3) from 13 unrelated patients, along with control samples (C1–C8), in a blinded fashion. Nine of the 10 BA-BP (herein referred to as "BA") specimens were skin lesions; all but 1 of these 9 patients were human

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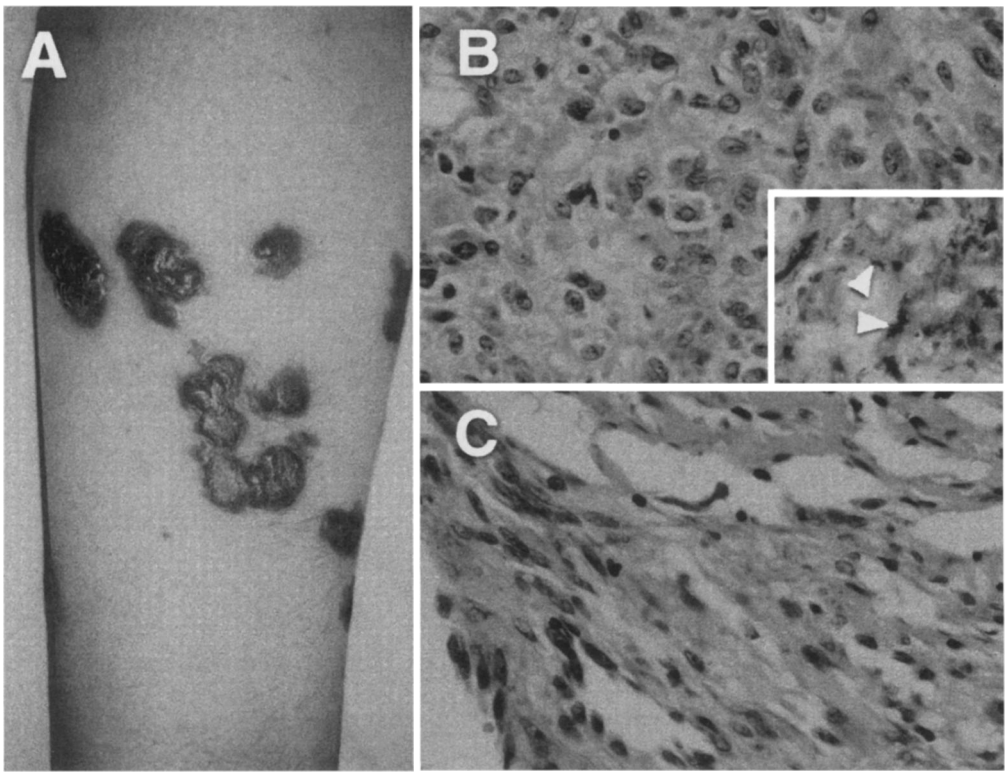

Figure 1. *A,* Close-up view of representative cutaneous lesions reveals well-demarcated violaceous plaques. Within plaques are irregularly arranged smooth, rounded red papules suggestive of bacillary angiomatosis/bacillary peliosis (BA). *B,* High-power view of superficial dermis from cutaneous biopsy specimen (hematoxylin-eosin stain) reveals lobulated vessels lined by plump, cuboidal endothelial cells, consistent with BA. Associated with superficial structures are amorphous clumps of lightly basophilic material. Warthin-Starry silver stain (insert) reveals aggregates of bacillary organisms (arrows). *C,* High-power view of deep reticular dermis from cutaneous biopsy specimen shows spindled cells forming irregular, slit-like vessels with extravasated red blood cells and hemosiderin, consistent with Kaposi's sarcoma.

immunodeficiency virus (HIV) seropositive. The HIV-seronegative patient with cutaneous BA was apparently immunocompetent and has been reported [3]. The tenth specimen (BA4) was spleen tissue with BP from an HIV-seropositive person. All 3 KS specimens were skin biopsies from HIV-seropositive patients. Eight coded "negative-control" samples were collected at the same institution as the BA and KS specimens and included 1 skin sample from an HIV-seronegative person (C1), 1 spleen sample from an HIV-seropositive person (C2), 2 spleen samples from HIV-seronegative persons (C3, C5), liver (C4) and bone (C6) from HIV-seropositive persons, a water sample (C7), and tissue digestion buffer (C8). All of the tissues listed were digested using a procedure described elsewhere [1] and then sent to one of the coauthors at a different institution for further analysis.

PCR assays. A PCR assay for 16S rDNA from *Bartonella* species and closely related α-proteobacteria was performed with primers 12E and 24B as described elsewhere [8]. All digested tissues were tested for human β-globin gene sequences with primers GH20 and PC04 using established conditions [8]. KSHV open-reading frame (ORF) 26 (minor capsid gene) DNA ($KS330_{233}$) was detected using two primers described by Chang et al. [7], KS-223F (5′-AGCCGAAAGGATTCCACCAT-3′) and KS-223R (5′-TCCGTGTTGTCTACGTCCAG-3′). The KSHV ORF 26 assay was done with the conditions described originally [7] as well as with the following modified conditions: 2 m*M* $MgCl_2$, 200 μM of each dNTP, 800 n*M* of each primer, 1 U of Amplitaq gold (Perkin-Elmer, Norwalk, CT), a 50-μL final reaction volume, 94°C incubation for 10 min, followed by 50 cycles comprising 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and then a 7-min incubation at 72°C. The sensitivity of the KSHV assay was determined using the modified set of conditions and serial dilutions of a plasmid containing a 600-bp fragment of ORF 26, which includes the two primer annealing sites (gift of D. Kedes and D. Ganem, University of California, San Francisco). In addition to controls C1–C8, negative controls included reactions with water and PCR reagents. All reactions were set up and analyzed in two different rooms; since BA, KS, and control specimens were analyzed in a blinded, coded fashion, positive and negative samples were randomly interspersed during analysis.

Case Report

A 37-year-old homosexual man with AIDS and 104 CD4 cells/mm^3 was diagnosed with KS 2 years before admission. He was undergoing treatment for cytomegalovirus (CMV) esoph-

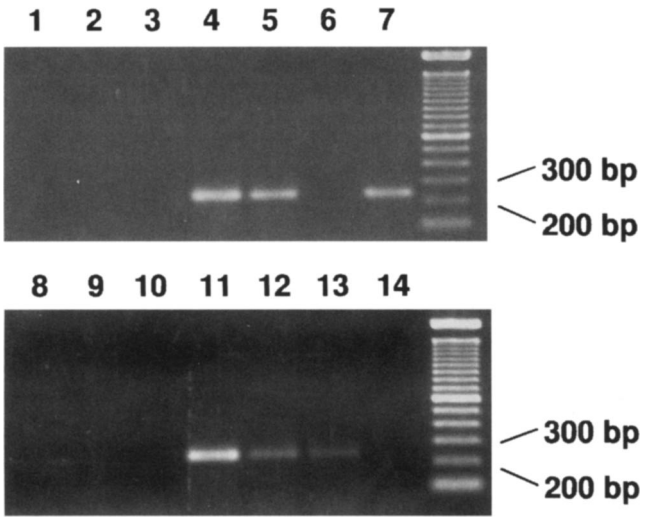

Figure 2. Agarose gel electrophoresis of DNA products from Kaposi's sarcoma–associated herpesvirus (KSHV) open-reading frame 26 polymerase chain reaction assay (using modified experimental protocol). By lane: 1, tissue BA1; 2, BA2; 3, BA3; 4, KS1; 5, KS2; 6, C1; 7, BA4, 8, water; 9, digestion buffer; 10, water; 11, 10^5 target copies; 12, 10^2 target copies; 13, 5 target copies; 14, 1 target copy. At right of each panel is 100-bp DNA ladder (200- and 300-bp bands indicated). Expected size of product from KSHV genome is 233 bp. BA, bacillary angiomatosis.

agitis and retinitis and diffuse cutaneous KS and was transfusion dependent. Three weeks before admission, he completed two cycles of bleomycin and vincristine. Subsequently, he developed daily fevers to 38°C and epigastric and right upper quadrant abdominal pain and was admitted for evaluation of CMV cholangitis. On admission, his temperature was 39°C. Physical examination revealed an enlarged liver and palpable spleen tip. Cutaneous findings included extensive, well-demarcated, violaceous cutaneous plaques. Localized and limited to and within these plaques were irregularly arranged smooth, rounded red papules and nodules, whose appearance was suspicious for BA (figure 1*A*). Skin biopsy specimens were obtained for histopathology and culture before initiation of antibiotics. The histopathologic findings revealed both KS and BA. In the superficial dermis (figure 1*B*), lobulated vascular structures lined by plump, cuboidal endothelial cells were found. Associated with the superficial vascular structures were amorphous clumps of lightly basophilic material, which were clumps of bacillary organisms on Warthin-Starry silver staining (figure 1*B* insert), consistent with BA. In contrast, in the deep reticular dermis (figure 1*C*), spindled cells formed irregular slit-like structures with extravasated red blood cells and hemosiderin consistent with KS.

B. henselae was isolated from the blood and from the cutaneous BA-KS lesion. On further questioning, the patient recalled having received a serious cat scratch 3 years earlier. The patient was treated with erythromycin but developed pleural effusion, pneumothorax, and acute renal failure and died on hospital day 17. An autopsy revealed disseminated BA, peliosis hepatitis, and generalized KS. Colocalization of BA and KS also occurred in the gastrointestinal tract and the lung. There was no evidence of cholangitis.

Results

Confirmation of diagnosis in BA and KS tissues. All 10 BA tissue digests yielded products of the expected size (296 bp) with the *Bartonella* range-restricted PCR assay, while none of the KS digests or controls generated any visible product (data not shown). *Bartonella* species (either *B. henselae* or *B. quintana*) were cultivated from some, and *Bartonella* species 16S rDNA was detected by amplification and DNA sequencing in all of the same BA specimens in previous experiments in a different laboratory [2]. A DNA product of the expected size (233 bp) was amplified from all 3 KS specimen digests by use of the KSHV PCR assay with both experimental protocols (see figure 2, lanes 4 and 5) and not from any digest buffer or PCR reagent-only controls (figure 2, lanes 8–10). By use of serial dilutions of cloned ORF 26 template DNA, the sensitivity of the KSHV PCR assay with the modified protocol was estimated to be 5 template copies (see figure 2, lanes 11–14).

All but 1 of the 19 tissue digests yielded an amplified product of the expected size using human β-globin gene primers on repeated testing over a 2-year period (data not shown); the exception (C1) was positive for β-globin DNA at the time of initial *Bartonella* and KSHV PCR testing; however, it was negative in the β-globin PCR assay 2 years later, when the modified KSHV assay was performed. These results provide evidence that all of the BA and KS tissues were infected with *Bartonella* species or KSHV, respectively, as one would have predicted, and that the tissue digests all contained amplifiable DNA.

Assessment of KSHV infection in BA and control tissues. No evidence of KSHV DNA was found in 9 of the 10 BA specimens. From specimen BA4, a product of the expected size was detected using the modified KSHV PCR assay (figure 2, lane 7). BA4 was a spleen sample with typical BP histology from an HIV-seropositive patient who had biopsy-proven cutaneous KS. Four of the 6 tissue controls with normal histology were negative in this KSHV PCR assay (C1, C3, C5, C6); tissues C2 and C4 were positive despite the absence of any pathology. C2 was splenic tissue from an HIV-seropositive person with intra-abdominal lymphatic KS. This result and that from BA4 might be explained by the known ability of KSHV to disseminate within the host well beyond sites of typical KS pathology [7, 9]. The other control tissue that yielded an unexpected positive result in the KSHV assay was a liver specimen (C4) with no evidence of KS from an HIV-seropositive person with angioimmunoblastic lymphadenopathy (AILD). In one study, KSHV DNA was detected in 3 of 15 tissue specimens with this

lymphoproliferative disease [10]. While our findings in this case and this study raise interesting questions about the possible role of this virus in AILD, we cannot exclude a noncausal association in a host at elevated risk for KSHV acquisition.

Discussion

KSHV has been associated with body cavity–based lymphoma, multicentric Castleman's disease, AILD, and KS. This virus encodes homologues of chemokine-like proteins vMIP-I and vMIP-II, which are angiogenic in vitro [11]. Our case and other similar cases have drawn attention over the past decade to the possibly frequent coassociation of KSHV and *Bartonella* species in BA lesions and a possible role for KSHV as a cofactor in the vascular proliferation of BA-BP. However, the results of our study argue against these possibilities. Our data indicate that if KSHV is found in BA lesions, it occurs infrequently or in extremely low numbers. These data suggest that KSHV is unlikely to be a cofactor in the vascular proliferation associated with BA bacilli and suggest that research efforts be focused on other possibilities. While the vascular proliferation in BA and BP may involve host induction or bacterial production of growth factors and cytokines that promote new vessel formation [12], the exact mechanisms of this fascinating and unusual host response to infection remain to be elucidated.

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