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Absence of Bacteria in the Temporal Arteries of Patients with Giant Cell Arteritis

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To the Editor

Giant cell arteritis (GCA) is the most common type of vasculitis to affect Caucasians over the age of 50 years.¹ Biopsy of a superficial temporal artery is commonly used to confirm the diagnosis of GCA. Although the pathophysiology of GCA has been extensively studied, the cause remains unknown. An infectious trigger for GCA has been considered but no organism has conclusively been linked to GCA.^{2,3} The presence of granulomatous inflammation and multinucleated giant cells in the temporal artery wall of some subjects led us to hypothesize that a bacterial infection causes GCA.⁴ Sensitive genomic sequencing techniques now make it possible to detect bacteria and other infectious organisms that were once unidentifiable by culture or other molecular techniques. To determine if GCA is caused by a bacterial infection, we cultured temporal arteries from subjects with GCA in media and analyzed the specimens daily for growth of bacteria. We also used 16S rRNA sequence technology to identify bacterial genomic sequences located in the temporal artery wall of subjects with GCA.

STUDY

This study was approved by the Institutional Review Boards of the University of Utah and the Department of Veterans Affairs Medical Center, Salt Lake City. Superficial temporal artery biopsy (STAB) samples were collected in accordance with the Declaration of Helsinki principles, and all subjects provided informed consent prior to participating in the study. Subjects with GCA met the 1990 American College of Rheumatology (ACR) criteria for GCA and had a STAB with histopathologic features consistent with GCA.⁵ STABs from subjects with clinical features of GCA but histopathologic features described by a pathologist as inconclusive were also analyzed. Control STABs came from subjects who were biopsied for suspicion of GCA, but they had histopathologically normal arteries and subsequent clinical follow up by a physician determined these patients to not have GCA. Temporal artery specimens were collected in the operating room using strict sterile procedures. A portion of the specimen was fixed in formalin, embedded in paraffin and sent for histopathologic analysis. Another portion of each specimen was placed into a sterile specimen container with or without RNALater^{TM} (Qiagen, Valencia, CA) and frozen at -80°C. Specimens were handled in a sterile tissue culture hood following standard microbiological and aseptic techniques. Investigators performing experiments were masked to the affected status of the samples analyzed.

Sections of STABs measuring approximately 1–2 mm were placed in sterile phosphate-buffered saline (PBS) and homogenized. A total of 20 µl of the homogenized suspension was plated onto Luria-Bertani (LB; Teknova, Hollister, CA), brain-heart infusion (BHI; Hardy Diagnostics, Santa Maria, CA) and chocolate media (Hardy Diagnostics, Santa Maria, CA). The remainder of the suspension was added to 3 ml of each LB (Gibco, Grand Island, NV) and BHI broth (Remel, Lenexa, KS). All cultures were incubated at 37° C with shaking for up to 14 days and checked daily for growth. Any observed colonies were isolated and characterized by traditional Gram stain and 16S rRNA sequencing.

DNA was isolated from bacterial colonies that grew on or in culture medium and from each STAB specimen using the DNeasy Blood and Tissue kit (Qiagen, Dusseldorf, Germany). Both protocols for isolating DNA from Gram-positive and Gram-negative bacteria were used according to the manufacturer's instructions.

DNA samples extracted from arteries and bacteria were used as a template in PCR reactions using 16S rRNA primers as described previously.^{6–8} Actin primers were used as an amplification control for human specimens.⁹ Genomic fragments amplified from temporal arteries were cloned into the pGEM T-easy vector following the manufacturer's instructions (Promega, Madison, WI). Genomic inserts were sequenced and compared to the Genbank Microbial database using Basic Local Alignment Search Tool (BLAST).

A total of 18 temporal arteries (10 histopathologic-proven GCA, 5 negative, and 3 indeterminate) were analyzed both by 16S rRNA sequencing and culture (Table 1). The 16S rRNA analysis of two PCR positive temporal arteries showed the sequences were not consistent with bacterial DNA but rather human DNA. Bacterial colonies grew on media inoculated with homogenized suspensions derived from three arteries (Table 1). 16S rRNA

sequence analysis of these colonies identified the bacterium in each case to be most consistent with *Staphylococcus epidermidis* (*S. epidermidis*).

CONCLUSIONS

We cultured samples from temporal arteries and used 16S rRNA sequence technology to determine if a bacterial infection causes GCA. *S. epidermidis*, a common skin contaminant, was isolated by culture from three artery samples but was not detectable in the temporal artery DNA by 16S rRNA PCR sequencing from the same subjects. For this reason as well as our inability to detect it by PCR, we are led to conclude that it is unlikely that *S. epidermidis* has a pathogenic role in GCA. 16S rRNA PCR sequencing did not identify any other bacterial sequences from the remaining 15 temporal arteries that were tested. These results suggest one of two possibilities, either bacteria do not reside in the temporal arteries of subjects with GCA or 16S rRNA sequencing is not sensitive enough to detect bacterial genomic material from temporal arteries.

Our study is limited by its small sample size. Furthermore, frozen temporal arteries were used for analysis rather than paraffin-embedded temporal artery tissue; however, some of the temporal arteries were stored in a solution to protect RNA. These solutions likely contain detergents that would kill or prevent growth of organisms that may reside in the tissue. While this preservative or our choice of culture media could reduce our ability to grow organisms in culture, it should not affect DNA extraction and PCR amplification of 16S rRNA from a bacterial pathogen. In our study, PCR using previously described 16S rRNA primers failed to detect bacterial genomic sequences from the temporal arteries of subjects with GCA. These findings differ from that of other investigators who have used different sequencing techniques to identify bacterial genomic fragments from arteries.^{2,10} These studies do not confirm that these fragments are from an organism that has a causal role in GCA. These sequences may be a remnant of a prior infection or secondary to contaminated PCR reagents. Others have recently shown that varicellazoster virus may play a causative role in GCA.¹¹ However, our experiments were not designed to detect most viruses or fungi in temporal arteries. Although this study does not rule out the possibility that a bacterium causes GCA, it does suggest that it is unlikely that GCA is caused by a live bacterial strain residing in the artery walls of these patients.

References

1. Salvarani C, Crowson CS, O'Fallon WM, et al. Reappraisal of the epidemiology of giant cell arteritis in Olmsted County, Minnesota, over a fifty-year period. *Arthritis Rheum.* 2004; 51:264–268. [PubMed: 15077270]
2. Gordon LK, Goldman M, Sandusky H, et al. Identification of candidate microbial sequences from inflammatory lesion of giant cell arteritis. *Clin Immunol.* 2004; 111:286–296. [PubMed: 15183149]
3. Bhatt AS, Manzo VE, Pdamallu CS, et al. In search of a candidate pathogen for giant cell arteritis: sequencing-based characterization of the giant cell arteritis microbiome. *Arthritis Rheumatol.* 2014; 66:1939–1944. [PubMed: 24644069]
4. Weyand CM, Younge BR, Goronzy JJ. IFN-gamma and IL-17: the two faces of T-cell pathology in giant cell arteritis. *Curr Opin Rheumatol.* 2011; 23:43–49. [PubMed: 20827207]
5. Hunder GG, Bloch DA, Michel BA, et al. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum.* 1990; 33:1122–1128. [PubMed: 2202311]

6. Mori H, Maruyama F, Kato H, et al. Design and Experimental Application of a Novel Non-Degenerate Universal Primer Set that Amplifies Prokaryotic 16S rRNA Genes with a Low Possibility to Amplify Eukaryotic rRNA Genes. *DNA Res.* 2014; 21:217–227. [PubMed: 24277737]
7. Greisen K, Loeffelholz M, Purohit A, et al. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. *J Clin Microbiol.* 1994; 32:335–351. [PubMed: 7512093]
8. Klausegger A, Hell M, Berger A, et al. Gram type-specific broad-range PCR amplification for rapid detection of 62 pathogenic bacteria. *J Clin Microbiol.* 1999; 37:464–466. [PubMed: 9889245]
9. Bhatt AS, Freeman SS, Herrera AF, et al. Sequence-based discovery of *Bradyrhizobium enterica* in cord colitis syndrome. *N Engl J Med.* 2013; 369:517–528. [PubMed: 23924002]
10. Haugebert G, Bie R, Nordbo SA. Temporal arteritis associated with *Chlamydia pneumoniae* DNA detected in an artery specimen. *J Rheumatol.* 2001; 28:1738–1739. [PubMed: 11469491]
11. Gilden D, White T, Khmeleva N, et al. Prevalence and distribution of VZV in temporal arteries of patients with giant cell arteritis. *Neurology.* 2015; 84:1948–1955. [PubMed: 25695965]

Table 1

Patient and sample characteristics

Subject	Histopathology of TA	Age	Gender	Bacterial growth on media	16S PCR of TA DNA	TA stored in RNA protectant solution
1	N	75	M	No	–	No
2	N	80	F	No	–	No
3	G	77	M	No	–	No
4	G	83	F	No	–	Yes
5	G	75	F	No	–	No
6	G	71	F	No	–	Yes
7	G	83	F	Yes	–	Yes
8	G	75	F	No	+	Yes
9	G	70	F	Yes	–	No
10	I	82	F	No	–	Yes
11	N	83	F	No	–	Yes
12	I	75	F	No	–	Yes
13	G	71	F	No	–	Yes
14	I	63	F	No	+	Yes
15	N	64	F	No	–	Yes
16	G	88	F	Yes	–	Yes
17	N	89	F	No	–	Yes
18	G	81	F	No	–	Yes

G: Histopathology of temporal artery consistent with giant cell arteritis (GCA)

N: Histopathology of temporal artery not consistent with GCA

I: Histopathology of temporal artery indeterminate for GCA

M: Male

F: Female

TA: Temporal artery

–16S PCR product not detected

+16S PCR product detected