Detection of Varicella Zoster Virus DNA in Some Patients with Giant Cell Arteritis

Bradley M. Mitchell¹,² and Ramon L. Font¹,³

PURPOSE. The purpose of this study was to determine whether an association exists between giant cell arteritis (GCA) and the presence of varicella-zoster virus (VZV), by using histologic, molecular, immunohistochemical, and ultrastructural analyses of temporal artery biopsy specimens.

METHODS. In a randomized masked study, 64 temporal artery biopsy specimens were analyzed by PCR for VZV DNA. The samples included 35 specimens histologically positive and 29 specimens histologically negative for GCA. Immunohistochemical staining for VZV viral antigen IE-63 was performed on seven of the specimens positive for GCA and five negative specimens. Transmission electron microscopy (TEM) was performed on five of the specimens positive for GCA.

RESULTS. PCR was positive for VZV DNA in 9 (26%) temporal arteries tested that showed histologic evidence of GCA. The remaining 26 histologically positive temporal arteries and all 29 histologically negative arteries tested gave negative PCR results for VZV DNA. Statistical analysis (z-test) comparing the association of VZV DNA between the specimens that were positive and negative for GCA showed a significant difference (P = 0.010). Immunohistochemical studies were positive in several biopsy specimens within adventitial histiocytes-macrophages, but these results did not correlate with either the presence or absence of VZV DNA or with the histologic evidence of GCA. No viral particles were observed by TEM.

CONCLUSIONS. This study showed a significant association of VZV DNA to temporal artery biopsy samples positive for GCA compared with the negative specimens. The results support the hypothesis that VZV may play a role in the pathogenesis of some cases of GCA. However, PCR, immunohistochemical, and electron microscopic findings suggest the virus is present at extremely low quantities, is abortively replicating, or is latent. (Invest Ophthalmol Vis Sci. 2001;42:2572–2577)

Giant cell arteritis (GCA) is a systemic vasculitis of unknown cause that involves predominantly the medium-sized arteries of the carotid circulation and sometimes the aorta and its branches. The main clinical findings are recent localized headaches, temporal artery tenderness, elevated erythrocyte sedimentation rate (ESR), and characteristic histologic findings on temporal artery biopsy. It may cause acute visual loss from ischemic optic neuritis or central retinal artery occlusion, with approximately half the subjects experiencing some degree of optic nerve involvement. Its origin is unknown, but viruses have been suspected as possible causative agents.

Varicella zoster virus (VZV), a member of the Herpesviridae family of viruses, is a potential candidate. It is a ubiquitous human pathogen, establishes a life-long latent infection from which it can reactivate, can be associated with arteries, and is capable of producing a granulomatous reaction with multinucleated cells.¹,² Similar to the incidence of GCA, the risk of VZV reactivation causing herpes zoster is age-associated. Approximately 75% of herpes zoster occurs in patients more than 45 years old,³ and the incidence increases to more than 10 cases per 1000 persons by the age of 75.⁴ A previous histopathologic study of 21 eyes enucleated for complications of herpes zoster ophthalmicus (HZO) revealed that two of the eyes had a granulomatous vasculitis involving the posterior ciliary arteries just behind the sclera.⁵ The histopathologic findings observed in these arteries showed a striking resemblance to those observed in cases of GCA. Additionally, another eye enucleated because of severe HZO disclosed diffuse granulomatous vasculitis involving the choroidal vessels. VZV has also been linked to generalized granulomatous angiitis of the central nervous system in immunocompromised patients,⁶,⁷ and detection of viruslike particles has been reported in granulomatous angiitis.⁸–¹⁰ These observations provide evidence that VZV may be associated with GCA.

The purpose of the present study was to further investigate whether VZV is associated with the pathogenesis of GCA. We histopathologically studied 71 temporal artery biopsy specimens, of which 37 were histologically positive and 34 were histologically negative for GCA. Nested PCR for VZV DNA (IE-63) was performed in a randomized masked study on DNA extracted from 64 specimens that were PCR positive for human β-actin and the findings compared with the results of the histopathology. Selected temporal artery biopsy specimens were also analyzed for viral antigen, by using immunohistochemistry and for viral capsids by using transmission electron microscopy (TEM). VZV-infected placental tissue and an eye enucleated because of HZO were evaluated as positive control specimens for the PCR and immunohistochemical studies.

MATERIALS AND METHODS

Sample Preparation and DNA Extraction

Seventy-one formalin-fixed, paraffin-embedded temporal artery biopsy specimens were studied retrospectively and prospectively. The samples included 37 arteries histologically positive for GCA and 34 histologically negative. Each artery was cross-sectioned at 1-mm intervals and embedded using the agar technique.¹¹ The embedded specimens were cut with a microtome at a thickness of 6 to 10 µm, using precautions necessary for PCR analysis. Four to six micrometre sections, each containing 6 to 10 pieces of temporal artery, were placed into sterile microfuge tubes. In a masked fashion, the DNA was extracted from each sample by using a protocol previously described,¹² with the
The deparaffinized sections were blocked with a 5% solution of normal goat serum followed by a 1:100 dilution of rabbit antiserum directed against the VZV IE-63 protein. Transmission Electron Microscopy

Formalin-fixed tissue from five temporal artery biopsy specimens that were PCR positive for VZV DNA was submitted for conventional TEM. Thick sections (≥1 μm) were stained with toluidine blue or paragon. Thin sections were stained with uranyl acetate and lead citrate and mounted on copper grids for examination.

RESULTS

Molecular Analyses

A nested PCR approach (Fig. 1) was used for screening temporal biopsy specimens. The first primer set used in the initial PCR reaction targeted nucleotides 110721 through 111106, which is an internal region of the IE-63 open reading frame (ORF). The second primer set used in the nested PCR reaction targeted nucleotides 110757 through 111082. The PCR assay was optimized using plasmid pVZV-EcoE, pUC19 containing the VZV EcoRI fragment from the Ellen strain. The first primer set amplified the predicted 386-bp region of pVZV-EcoE (Fig. 2). The second primer set, using 20% of the first PCR reaction mixes as a template, produced a 326-bp PCR product. The second (nested) PCR increased the lower limit of detection (Fig. 2). Although the band intensity of the lower limit of detection varied, the nested PCR consistently detected less than 10 copies of pVZV-EcoE.

Specificity of the PCR assay was determined using regions of VZV DNA cloned into plasmids and viral genomic DNA isolated from several closely related members of the Herpesviridae family as templates. Plasmid pVZV-EcoE, as expected, was readily detected, whereas pVZV-EcoB, a plasmid containing the EcoRI B fragment of VZV (Fig. 1) gave negative PCR results. The PCR assay did not amplify a detectable product using as much as 1 ng of genomic DNA isolated from strains of HSV-1 (KOS and 17+syn), HSV-2 (HG52), or MCMV.
DNA was extracted from 71 masked samples of temporal artery biopsy specimens and tested in control β-actin PCR assays. Sixty-four samples were confirmed to be acceptable for PCR analysis, of which 35 were histologically positive for GCA and 29 were negative. The β-actin–positive samples were analyzed using the nested VZV PCR assay (Table 1). PCR was positive for VZV DNA in 9 (26%) temporal artery specimens that showed histologic evidence of GCA. The remaining 26 histologically positive temporal arteries and all 29 histologically negative arteries gave negative PCR results for VZV DNA. The positive results were confirmed by repeated PCR analysis. Statistical analysis (z-test) comparing the association of VZV DNA between the specimens positive and negative for GCA showed a significant difference (P = 0.010). The samples were unmasked. Representative PCR results are shown in Figure 3.

VZV IE-63 Immunohistochemical Analysis

Detection of VZV DNA within temporal artery biopsy specimens provided evidence for the presence of virus in some of the samples examined. Immunohistochemical analysis was used in an attempt to identify and localize VZV viral antigen in situ. Positive immunoreactivity was observed in approximately half the specimens and restricted to adventitial histiocytes-macrophages (Fig. 4). Scattered immunoreactive cells showed granular cytoplasmic deposits in the adventitial histiocytes. In an unexpected finding, the staining was not unique to temporal arteries positive for GCA but was also present in some arteries that were histologically negative for GCA.

TEM Analysis

Previous studies have reported viruslike particles in granulomatous angiitis of the central nervous system.8–10 Consistent with our immunohistochemical findings, one of these studies also presented results of antigen related to VZV in the nuclei and cytoplasms of histiocytes in a case of a granulomatous angiitis from the basilar artery.10 This prompted us to perform ultrastructural analysis on several of our temporal artery biopsy specimens that were histologically positive for GCA and positive or negative for VZV DNA by PCR analysis. We carefully screened all sections of the artery with emphasis on the adventitial mononuclear cells and histiocytes. Because of the potential for VZV to spread to arterial walls by neural pathways,23 neuronal tissue within the biopsy specimens was also closely examined (Fig. 5). No viral particles were observed in TEM evaluation.

DISCUSSION

Giant cell arteritis is a chronic granulomatous inflammation involving medium-sized arteries of elderly individuals. There is ocular involvement, ranging from diplopia or transitory vision loss to complete blindness, in approximately half the subjects. Although it has been proposed to be an autoimmune disease,24 the actual cause is unknown.
Application of the highly sensitive and specific PCR-based technique described herein to temporal artery biopsy specimens demonstrated an association of VZV DNA and GCA. Viral DNA was present in 26% of the specimens histologically positive for GCA. Although this association was statistically significant, VZV DNA was not detected in 74% of the specimens from this group. A possible explanation for this finding is that VZV may be associated with only a subset of cases of GCA. Another reason may be that viral DNA was present at amounts below the level of detection. The primers used for PCR amplification target a conserved region of the VZV genome and have been shown previously to amplify a wide variety of clinical VZV isolates. Therefore, a negative result is not likely to be a false negative, because of viral strain genetic variability. Regardless, VZV DNA was amplified from approximately one quarter of the temporal artery biopsy specimens that were histologically positive for GCA.

Whether the association of VZV DNA and GCA is causal or casual cannot be determined from our findings. It is plausible that GCA and its associated granulomatous inflammation are
related to the VZV DNA’s becoming detectable. Another possibility is that the VZV DNA is only incidentally associated with GCA.

Because VZV DNA was present in a significant portion of the temporal artery biopsy specimens tested, it was of interest to determine whether viral antigen or viral particles could be detected, regardless of the type of association that might exist. Our immunohistochemical findings of positive immunoreactivity of histiocytes-macrophages was consistent with a report that used antiserum obtained from patients who had herpes zoster, one of whom had a basilar artery aneurysm at the site of granulomatous angiitis. Of interest, the positive immunoreactivity we observed was not unique to temporal arteries positive for GCA but was also present in some arteries that were histologically negative for GCA or were negative by PCR analysis.

Possible reasons for the discordance between our PCR and immunohistochemical results may include issues of sensitivity or sampling problems within the biopsy; however, we do not believe that these adequately explain our findings. It is unlikely that the immunohistochemical staining would be more sensitive than PCR, especially at the lower limit of detection that we have demonstrated for the PCR assay. Also, because the sections used for immunohistochemical staining were serial sections to those used for PCR analysis, we believe sampling problems within the biopsy specimens were not likely, especially when considering that multiple specimens gave similar results. A more likely explanation is that the primary antibody used in the immunohistochemical staining cross-reacted with some antigen on the adventitial histiocytes. A similarity between the study by Fukumoto et al. and ours is that they both showed positive staining with granular cytoplasmic deposits within the adventitial histiocytes. The report by Fukumoto et al. did not include control tissue, and the specificity of the immunoreactivity they reported is therefore unclear.

One difference between our findings and those of Fukumoto et al. is the presence of viral particles. Whereas they demonstrated enveloped viral particles in the cytoplasm of the histiocytes in the walls of the aneurysm by using electron microscopy, we did not detect any viral particles, even after extensive evaluation of several different specimens. This could have been due to a difference in the prevalence of virus in temporal arteries compared with the basilar artery lesion evaluated and even suggests that viral particles may be absent during the clinical manifestations of GCA. The absence of viral particles would not necessarily exclude the potential presence of viral DNA or antigens. For instance, viral DNA and some viral antigens, including the protein IE-63, are present during latent infections, but viral replication and the production of virus particles are limited.

Contrary to our findings of VZV’s association with GCA are the results of Nordborg et al. who reported the inability to detect either VZV antigen or DNA in arteries from 10 histopathologically verified cases of GCA. Final conclusions about these negative findings should be made with caution because of the potential limitation in the sensitivity of the PCR assay used and the small sample size studied. The PCR assay used by Nordborg et al. involved a single PCR reaction and detected 10 copies of a plasmid containing VZV DNA. This lower limit of detection was similar to our experience using a single-PCR–reaction approach (data not shown); however, we were able to increase our lower limit of detection by approximately two logs by using the double-PCR assay described. Secondly, at an expected prevalence of 26%, twice the number of specimens tested by Nordborg et al. would be needed to assure at least one positive result at a confidence level of 0.8 (α = 0.05). Therefore, either the lower limits of detection or the number of specimens evaluated in the two studies could account for the ultimate differences in the results obtained.

The granulomatous vasculitis typically associated with multinucleated giant cells, which is histologically characteristic of GCA, is consistent with an infectious cause. The fact that GCA is an arthritic disease and that it is associated with the elderly are both consistent with VZV as the responsible agent. Findings in our study further support the hypothesis that VZV may play a role in the pathogenesis of some cases of GCA. These results provide some of the first direct data that VZV may be a causative agent for GCA. However, our immunohistochemical and electron microscopic findings suggest the virus was present at extremely low quantities, abortively replicating, or latent.
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References