RESEARCH LETTER

Varicella-Zoster Virus Expression in the Cerebral Arteries of Diabetic Subjects

Primary varicella-zoster virus (VZV) infection causes varicella (chickenpox), after which VZV becomes latent in ganglionic neurons along the entire neuraxis. A decline in cell-mediated immunity to VZV in elderly and immunocompromised individuals results in zoster (shingles). Within the first year after herpes zoster, there is a 30% increased risk of stroke.\(^1\,2\) Approximately one-third of patients with VZV vasculopathy do not have zoster rash and diabetic patients are at greater risk for both zoster\(^3\) and stroke; therefore, we examined the cerebral arteries of 4 diabetic subjects for the presence of VZV DNA and antigen.

Methods. Cerebral arteries obtained from 4 subjects with diabetes less than 24 hours after they died were analyzed. All subjects had diabetes and no history of zoster infection, transient ischemic attacks, stroke, or immunosuppression. Subject 1 was a 51-year-old woman with hypertension who died of drug overdose; subject 2 was a 60-year-old man with systemic lupus erythematosus who died of asphyxia; subject 3 was a 51-year-old woman who died of myocardial infarction; and subject 4 was a 60-year-old woman with melanoma, hypertension, and hyperlipidemia who died of a drug overdose. An average of 17 pieces of cerebral arteries (about 10 mm/piece) from each subject were analyzed (total of 68). Each piece was cut in half. DNA was extracted from the first half and analyzed by real-time polymerase chain reaction for the presence of VZV DNA using VZV gene 63 specific primers (gene 63-forward 5'-GCTTACGCGCTACTTTAATGGAA-3' and gene 63-reverse 5'-GCCTCAATGAACCCGTCTTC-3') and probes (gene 63 5'-TGTTCCCATCGACCCCTCGG-3').

Figure 1. Histological examination of the varicella-zoster virus (VZV)–positive cerebral artery from neurologically asymptomatic diabetic subject 3. A, A hematoxylin-eosin–stained section of region 1 of VZV DNA–positive artery showed inflammation restricted to the arterial adventitia (arrow). B, Incubation of the adjacent section with rabbit anti-VZV gene 63 antibodies\(^6\) revealed VZV antigen in the adventitia of 5 consecutive arterial sections spanning 30 µm (arrows) that was not seen with control normal rabbit serum (C). D, A hematoxylin-eosin–stained section of region 2 approximately 150 µm downstream from region 1 of the VZV DNA–positive artery revealed minimal inflammation and no staining with rabbit anti-VZV IgG antibody (E) or control normal rabbit serum (F). G, A hematoxylin-eosin–stained section of region 3 approximately 150 µm downstream from region 2 again showed inflammation in the adventitia. Region 3 was positive for VZV antigen staining in all 10 sections spanning 60 µm (arrow) but not with control normal rabbit serum (I), indicating that VZV infection in the artery was not contiguous (original magnification \(\times 200\)).
Positive controls were provided by amplification of serial dilutions of known quantities of VZV DNA. Negative controls were provided by omission of VZV DNA from the polymerase chain reaction, which was performed 3 times. The other half was formalin-fixed and paraffin-embedded. If VZV DNA was detected, immunohistochemical analysis was performed on the remaining formalin-fixed, paraffin-embedded arterial sections for VZV gene 63 protein and leukocyte CD45 antigen. Limited arterial tissue precluded a search for additional antigens. Slides were viewed by Nikon Eclipse E800 microscopy with AxioVision digital imaging software (Carl Zeiss MicroImaging GmbH).

Results. Multiple cerebral arteries from 4 diabetic subjects were analyzed for the presence of VZV DNA. Because 1 of 17 arterial pieces from subject 3 was found to contain 3740 copies of VZV DNA per microgram of total DNA, multiple 6-µm sections were prepared from the corresponding formalin-fixed, paraffin-embedded piece for histological and immunohistochemical analysis. The 1 piece was then analyzed histologically by hematoxylin-eosin staining and for VZV gene 63 protein and CD45 antigen expression. Hematoxylin-eosin staining revealed inflammation in the arterial adventitia (Figure 1A, D, and G), mostly in region 1. Cells containing VZV antigen were seen in noncontiguous regions 1 and 3 separated by 300 µm (Figure 1B and H); the intervening region 2 did not contain VZV antigen (Figure 1E). Staining with control normal rabbit serum was negative in all 3 regions (Figure 1C, F, and I). Leukocytes in region 1 that expressed CD45 antigen (Figure 2B) were associated with cells containing VZV antigen (Figure 2A). Varicella-zoster virus antigen and cells expressing CD45 antigen were also found in region 3 but not in region 2 (Figure 2C and D).

Comment. Varicella-zoster virus infection of a cerebral artery in the absence of neurologic symptoms related to that artery was first found in the temporal artery of a patient with VZV vasculopathy. Extension of asymptomatic VZV infection of a cerebral artery in a diabetic subject raises several issues. First, because strokes are common in people with diabetes and attributed to atherosclerosis, it may be prudent to consider VZV vasculopathy as an additional cause of stroke in people with diabetes, particularly since VZV vasculopathy is treatable with antiviral drugs. Second, VZV antigen was found in noncontiguous arterial regions, underlining the need to examine multiple areas of a single artery to identify virus, just as multiple areas are examined to diagnose giant cell arteritis. Third, the detection of VZV exclusively in arterial adventitia supports earlier observations that VZV vasculopathy begins in the adventitia, most likely after transaxonal spread of virus from ganglionic afferent fibers. Finally, while more diabetic subjects require study, identification of cells expressing CD45 in proximity to cells containing VZV antigen points to the need for further analysis of the immune repertoire in cerebrovascular remodeling and stroke.

Maria A. Nagel, MD
Igor Traktinskiy, BS
Alexander Choe, BA
April Rempel
Don Gilden, MD

Author Affiliations: Departments of Neurology (Drs Nagel and Gilden, Messrs Traktinskiy and Choe, and Ms Rempel) and Microbiology (Dr Gilden), University of Colorado School of Medicine, Aurora.

Correspondence: Dr Nagel, Department of Neurology, University of Colorado School of Medicine, PO Box B182, 12700 E 19th Ave, Aurora, CO 80045 (maria.nagel@ucdenver.edu).

Author Contributions: Study concept and design: Nagel and Gilden. Acquisition of data: Nagel, Traktinskiy, Choe, Rempel, and Gilden. Analysis and interpretation of data: Nagel and Gilden. Drafting of the manuscript: Nagel and Gilden. Critical revision of the manuscript for important intellectual content: Nagel, Traktinskiy, Choe, and Rempel. Obtained funding: Nagel and Gilden. Administrative, technical, and material support: Nagel, Traktinskiy, Choe, and Rempel. Study supervision: Nagel and Gilden.

Financial Disclosure: None reported.

Funding/Support: This study was supported by National Institutes of Health grants NS067070 (Dr Nagel), AG032958, and AG006127 (Dr Gilden). Ms Rempel’s work was supported by National Institutes of Health grant 5R25GM083333.

Additional Contributions: We thank Marina Hoffman, BA, for editorial assistance and Cathy Allen for manuscript preparation.

Methodological Issues With the Risk of Relapse Study in Patients With Multiple Sclerosis After Yellow Fever Vaccination

We note methodological issues with the study by Farez and Correale. They observed relapses of multiple sclerosis (MS) in 5 of 7 patients with MS after yellow fever (YF) vaccination and used a self-controlled case series method to elucidate a possible association. They also selected matched controls among healthy subjects and patients with MS vaccinated with inactivated influenza vaccine for comparison of immunological responses to vaccination.

The most important assumption of the self-controlled case series method is that exposures are independent of earlier events. This assumption is required for the conditioning argument by which the case series likelihood is derived. However, the decision to administer YF vaccination clearly is not independent of the presence of, or risk for, MS relapse. Travel to endemic areas is least likely during or soon after an MS relapse. Accordingly, the assumption that exposure (vaccination) is independent of earlier events (eg, latest relapse) is not valid. Moreover, as time passes, symptoms remit and likelihood of travel increases, but equally, the (unknown) time of next relapse also draws nearer. Thus, exposure (vaccination) is also associated with likelihood of the next event occurring within any defined postexposure window, independent of the presence or absence of a causal association between exposure and the event. These biases invalidate the analysis. Such bias is less of an issue with vaccines that are mandated at certain ages or used seasonally but still needs to be tested for (Whitaker et al provide a motivating example of idiopathic thrombocytopenic purpura and measles, mumps, and rubella vaccine).

Farez and Correale also compared immunological changes after YF vaccination with those after inactivated influenza vaccine and also in healthy unvaccinated controls. The differences noted are to be expected when comparing recipients of a live vaccine vs an inactivated product or no vaccine at all and have no specificity for YF vaccination. Finally, the analysis of magnetic resonance imaging changes after vaccination is unreliable because of the earlier-discussed bias and also because the controls were not matched on time since onset or severity of the disease.

We conclude that the inference of causal relationship based on “temporal relationship between events” and “strength of exacerbations” is not supported owing to invalid study design. What remains is an argument of biological plausibility—the weakest line of evidence.

Vitalli Pool, MD
Daniel M. Gordon, MD
Michael Decker, MD, MPH

Author Affiliations: sanofi pasteur, Swiftwater, Pennsylvania.
Correspondence: Dr Pool, Discovery Drive, Swiftwater, PA 18370 (vitalii.pool@sanofipasteur.com).
Author Contributions: Study concept and design: Pool. Analysis and interpretation of data: Pool, Gordon, and Decker. Drafting of the manuscript: Pool. Critical revision of the manuscript for important intellectual content: Gordon and Decker. Statistical analysis: Pool. Study supervision: Gordon and Decker.
Financial Disclosure: Drs Pool, Gordon, and Decker are employees of sanofi pasteur.