Congenital Varicella-Zoster Virus Infection and Barrett's Esophagus

Xilla T. Ussery,* Paula Annunziato, Anne A. Gershon, Barbara S. Reid, Octavian Lungu, Claire Langston, Saul Silverstein, Kenneth K. Lee,* and Carol J. Baker

Departments of Pediatrics, Microbiology and Immunology, and Pathology, Baylor College of Medicine, Houston, Texas; Department of Pediatrics and Microbiology, Columbia University College of Physicians and Surgeons, New York, New York

Congenital varicella syndrome is a rare complication of varicella-zoster virus (VZV) infection during pregnancy. An infant was exposed to VZV at 18.5 weeks of gestation and had eye and skin abnormalities at birth and persistent feeding difficulties, prompting esophageal biopsies at 12 days and 20 and 20.5 months of age. Esophageal tissues demonstrated specialized intestinal metaplasia (Barrett's esophagus). VZV DNA (in situ hybridization) and proteins (immunohistochemistry and polymerase chain reaction) were found in esophageal epithelial cells adjacent to the Barrett's lesion. Immediate-early 63 protein (IE63) of VZV was demonstrated in the day 12 specimen, and IE62 and the late VZV glycoprotein E (gE) were found in the 20-month specimen. Clinical and endoscopic improvement followed fundoplication and acyclovir therapy, but VZV DNA and IE62 persisted in esophageal tissue. These findings associate VZV with specialized intestinal metaplasia within the esophagus and suggest a novel site for either latent or active VZV infection.

Primary varicella-zoster virus (VZV) infection during pregnancy can result in a spectrum of outcomes affecting both the mother and fetus. In the mother, infection may be mild or complicated by pneumonia and even death. When infection occurs during the first trimester, there is a 1%–9% chance that the fetus will develop congenital varicella syndrome [1]. This syndrome is characterized by cicatricial skin scarring, eye and brain abnormalities, and limb hypoplasia. Several other abnormalities, including swallowing dysfunction and aspiration pneumonia, also have been reported [2]. We describe the first case of congenital varicella syndrome associated with specialized columnar epithelium with intestinal metaplasia in the esophagus, commonly called Barrett's esophagus [3]. We used in situ hybridization and immunohistochemistry to demonstrate VZV DNA and proteins in esophageal tissue adjacent to the Barrett's lesion found at 12 days and 20 and 20.5 months of age.

Case Report

Antenatal history. This was the second pregnancy for a 34-year-old woman without a history of VZV infection or VZV-specific antibodies. At 18.5 weeks of gestation, she was exposed to her 5-year-old daughter with chickenpox and received varicella zoster immune globulin 2 days later. Nine days after onset of the daughter's rash, the mother developed varicella. There were no other complications or illnesses during her pregnancy.

Postnatal history. The infant was a 2780-g (10th percentile) white girl, born at 40 weeks of gestation by spontaneous vaginal delivery. Her Apgar scores were 7 and 8 at 1 and 5 min, respectively. Feeding intolerance became apparent on day 1. On day 3, barium swallow revealed severe gastroesophageal reflux, uncoordinated swallowing, significant aspiration, and an area of narrowing and irregularity in the distal esophagus. Esophagogastroduodenoscopy on day 12 showed circumferential exudative inflammation and edema of the distal 2 cm of the esophagus, corresponding with the radiographically abnormal region. This area was above the lower esophageal sphincter (LES) and diaphragmatic pinch. The Z-line or squamocolumnar junction could not be identified endoscopically, but there were no gastric rugal folds in this area. Neurologic examination was normal. The patient was treated with ranitidine, cisapride, and formula feedings via nasogastric tube. However, feeding difficulty persisted and cine esophagram on day 31 revealed extreme esophageal dysmotility. The infant underwent gastrostomy tube placement and fundoplication on day 37.

On day 38, ophthalmologic examination revealed bilateral macular chorioretinal lesions with anomalous vasculature, optic disk pallor, and a nuclear cataract on the right. Also, she developed 3–4 erythematous-based vesicular lesions on the right thigh, from which VZV was subsequently isolated, and several faint 2- to 3-mm hypopigmented lesions were noticed on the trunk and extremities that the mother recalled having been present since birth. Serology for human immunodeficiency virus (HIV) was negative. High titers of IgG antibody to VZV were detected in maternal and infant sera. The infant was treated with intravenous acyclovir (30 mg/kg/day) for 7 days.

The infant was discharged at 7 weeks of age and readmitted on multiple occasions for lower respiratory infections. She had recurrent episodes of retching associated at times with guaiac-positive ''coffee-ground'' material from the gastrostomy. At 20 months of age, she was hospitalized for bronchopneumonia and

Received 22 November 1997; revised 18 March 1998.

Financial support: NIH (AI-12402 to A.A.G. and S.S.); VZV Research Foundation (to O.L.).

Reprints or correspondence: Dr. Carol J. Baker, Baylor College of Medicine, Department of Pediatrics, One Baylor Plaza, Room 307E, Houston, TX 77030 (cbaker@bcm.tmc.edu).

* Present affiliations: East Tennessee Children's Hospital, Pediatric Infectious Diseases, Knoxville (X.T.U.); University at Buffalo, State University of New York, Department of Pediatrics, Division of Gastroenterology and Nutrition, School of Medicine and Biomedical Sciences, Children's Hospital, Buffalo, New York (K.K.L.).

The Journal of Infectious Diseases 1998; 178:539–43

0022–1899/98/7802–0033$02.00

hematemesis. She underwent repeat upper endoscopy and biopsy. The distal 2 cm of esophagus was erythematous and friable; white exudate was visible 3–4 cm above the LES. Intravenous acyclovir (30 mg/kg/day) was begun, and the child underwent repeat fundoplication. Three days after the completion of 10 days of acyclovir, a third endoscopy was done. The esophageal mucosa appeared normal, although the abnormal mucosa noted prior to surgery was in the area of the fundoplication wrap and could not be visualized.

At 38 months of age she is blind but has no other neurologic impairments, and developmental testing is consistent with a functional age of 30 months.

Methods

The child underwent endoscopy at 12 days and 20 and 20.5 months of age, and biopsies were obtained with careful attention to their relationship to the LES. Tissue from the second and third endoscopy procedures was cultured for viruses. The biopsy specimens were fixed in formalin and embedded in paraffin for routine histopathologic examination and staining with alcian blue at pH 2.5.

Sections of paraffin-embedded esophageal biopsy specimens obtained at 12 days and 20 and 20.5 months of age were prepared for in situ hybridization, immunohistochemistry, and polymerase chain reaction (PCR) following previously published methods [4]. In situ hybridization for VZV was done using a fluorescein-labeled oligonucleotide probe complementary to 27 internal base pairs of the VZV open-reading frame 54, with the sequence CCFGATGCAACFGTTGGFCCAGAGGAGCFGG, where F denotes fluorescein. To control for nonspecific hybridization, in situ hybridization using a herpes simplex virus type 1 (HSV-1) probe also was done [5].

Immunohistochemical analysis was done after treating the slides with Serotec Target unmasking fluid (Harlan Bioproducts for Science, Indianapolis) according to the manufacturer's recommendations. The VZV immediate-early proteins, IE62 and IE63, were detected by rabbit anti-IE62 (provided by I. Hay, State University of New York, Buffalo) and rabbit anti-IE63 (provided by B. Rentier, University of Liège, Liège, Belgium), and visualized by fluorescein-conjugated anti-rabbit secondary antibody (Boehringer Mannheim, Indianapolis). We further amplified the signal with anti-fluorescein alkaline phosphatase–conjugated antibody as previously described [4]. The VZV glycoprotein gE was detected with fluorescein-conjugated anti-gE antibody (Ortho Diagnostics, Raritan, NJ).

Results

The esophageal biopsy on day 12 was taken from the area of circumferential inflammation within the distal 2 cm of esophagus, and provided 3 mucosal fragments for histologic evaluation. Two of these revealed basal hyperplasia with moderate neutrophilic infiltrate without eosinophils, consistent with acute esophagitis. In these 2 fragments, VZV DNA was detected by in situ hybridization in the nuclei of epithelial cells. Immunohistochemical analysis of these fragments detected nuclear and cytoplasmic VZV IE63 (figure 1A, B). The third esophageal mucosal fragment showed villiform mucosa resembling small intestine with columnar epithelium and goblet cells; there was focal surface erosion and marked acute inflammation. The changes were consistent with specialized intestinal metaplasia of the esophagus (figure 2A). This fragment was negative for VZV by both in situ hybridization and immunohistochemistry for IE63 (figure 1C and D).

At 20 months of age, the infant underwent a second endoscopic procedure, and biopsies were taken 2 cm, 3–4 cm, and 5–6 cm proximal to the LES. Histology of the most distal biopsy revealed chronically inflamed squamous mucosa with a central area of glandular epithelium with goblet cells (figures 2B and C). VZV DNA again was detected in the nuclei of epithelial cells by in situ hybridization; immunohistochemistry detected IE62 and gE in the nuclei and cytoplasm of many epithelial cells (figure 1E–G). After fundoplication and acyclovir treatment, a third endoscopy was done at age 20.5 months. Biopsies taken within 3 cm of the surgically altered LES, representing mucosa $\geqslant$4 cm above the esophagogastric junction, revealed mild chronic inflammation consistent with a resolving inflammatory process (figure 2D). VZV DNA was detected in the nuclei of several epithelial and submucosal cells; immunohistochemistry detected only IE62 and not gE in the nuclei and cytoplasm of epithelial cells (figure 1H–J).

In situ hybridization for HSV-1 was negative for all 3 esophageal biopsies. PCR of the esophageal fragments for VZV and for the human β-globin gene were negative, indicating that DNA extracted from this specimen could not be amplified. Viral culture of tissues obtained on the second and third endoscopies also was negative.

Discussion

Infection with VZV commonly occurs in childhood, with 70%–80% of young adults in the United States reporting a history of chickenpox. It is estimated that $<$25% of adults without a history of chickenpox are susceptible to VZV infection [6]. Unfortunately, there are few data regarding the incidence of VZV infection and congenital varicella syndrome during pregnancy. Current studies estimate the risk of congenital varicella syndrome to be $<$10% if infection occurs during the first trimester [1]. By all accounts, it is a rare occurrence and to date, $<$100 cases have been described. This is one of the first reports of congenital varicella syndrome with tissue confirmation of the diagnosis.

Our patient had classic manifestations of congenital varicella syndrome, including eye and skin abnormalities. In addition, zoster, documented by culture, appeared at 5.5 weeks of age. Although esophageal dysmotility has been reported in infants with congenital VZV syndrome [2], and VZV infection of the esophagus has been reported [7], specialized columnar epithelium with intestinal metaplasia, also called Barrett's esophagus,

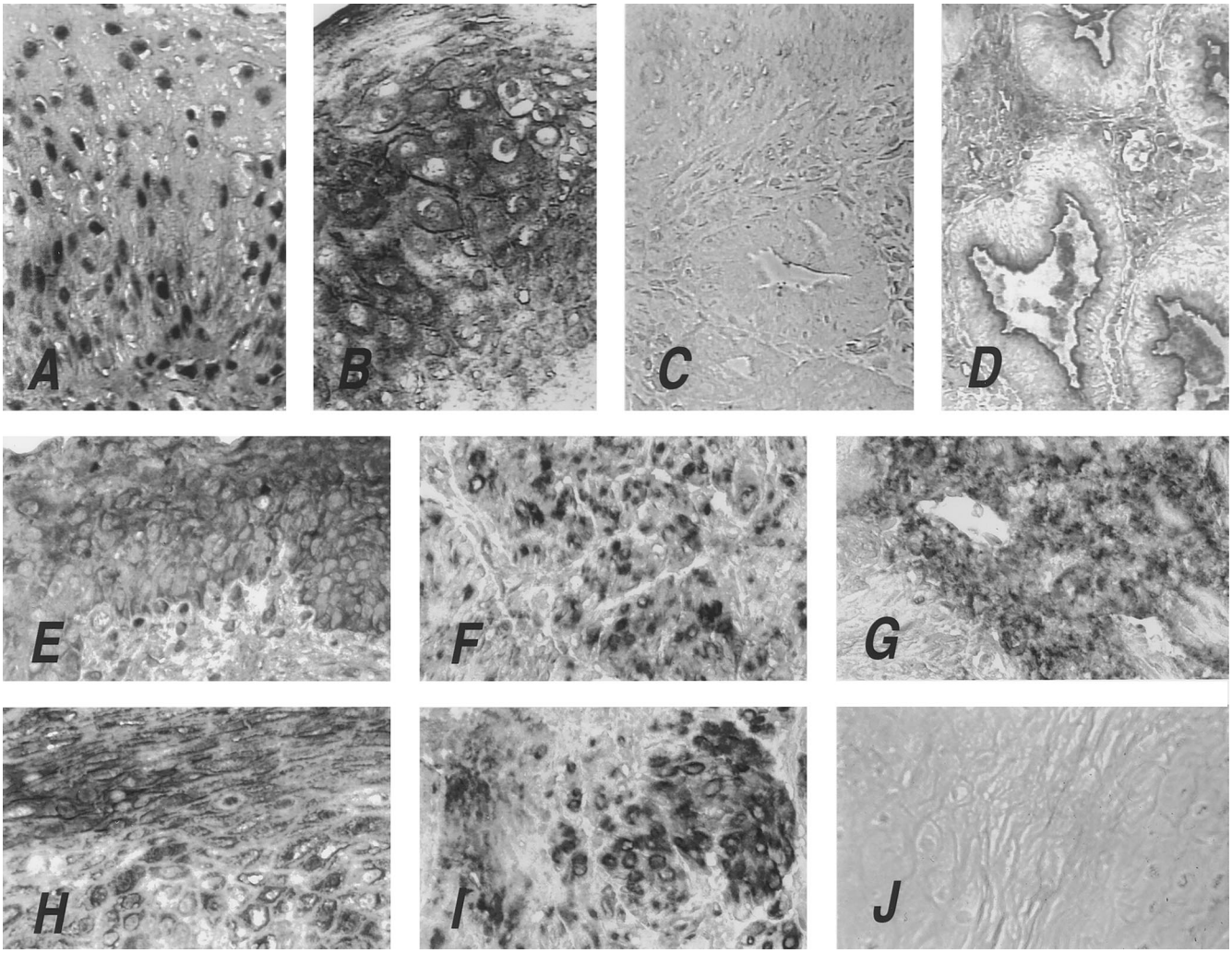

Figure 1. In situ hybridization and immunohistochemical analysis of VZV in esophageal biopsies. **A**, Biopsy at 12 days of age. VZV DNA in nuclei of majority of epithelial cells is detected by in situ hybridization. **B**, Biopsy at 12 days of age. VZV IE63 in nuclei and cytoplasm of epithelial cells is detected by immunohistochemistry. **C**, Biopsy at 12 days of age from site of glandular metaplasia. VZV DNA is not detected in epithelial cells by in situ hybridization. **D**, Biopsy at 12 days of age from site of glandular metaplasia. VZV IE63 is not detected in epithelial cells by immunohistochemistry. **E**, Biopsy at 20 months of age before acyclovir treatment. VZV DNA in nuclei of few epithelial and submucosal cells is detected by in situ hybridization. **F**, Biopsy at 20 months of age before acyclovir treatment. VZV IE62 in nuclei and cytoplasm of many epithelial cells is detected by immunohistochemistry. **G**, Biopsy at 20 months of age before acyclovir treatment. VZV gE in nuclei and cytoplasm of epithelial cells is detected by immunohistochemistry. **H**, Biopsy at 20.5 months after acyclovir treatment. VZV DNA in nuclei of several epithelial and submucosal cells is detected by in situ hybridization. **I**, Biopsy at 20.5 months of age after acyclovir treatment. VZV IE62 in nuclei and cytoplasm of many epithelial cells is detected by immunohistochemistry. **J**, Biopsy at 20.5 months of age after acyclovir treatment. VZV gE is not detected in epithelial cells by immunohistochemistry.

associated with VZV infection previously has not been reported.

Accurate diagnosis of specialized columnar epithelium with intestinal metaplasia in the esophagus depends on careful documentation of the localization of the pathology [8]. Although the Z-line in our patient was obscured because of mucosal inflammation, the biopsy sites were above the diaphragmatic hiatus in an area without gastric rugal folds that corresponded to the abnormal area on barium esophagram. Biopsies taken at the same level of the tubular esophagus demonstrated both squamous epithelium and columnar epithelium with intestinal metaplasia. These histologic findings were found again at repeat endoscopy 19.5 months later.

Although the diagnosis of specialized columnar epithelium in the esophagus is more common in adults, it has been reported in infants and children. These histologic changes are usually associated with long-standing gastroesophageal reflux [9]. Two explanations concerning the pathogenesis of VZV-associated Barrett's esophagus appear plausible. First, the esophageal findings may be due to direct epithelial injury from VZV infec-

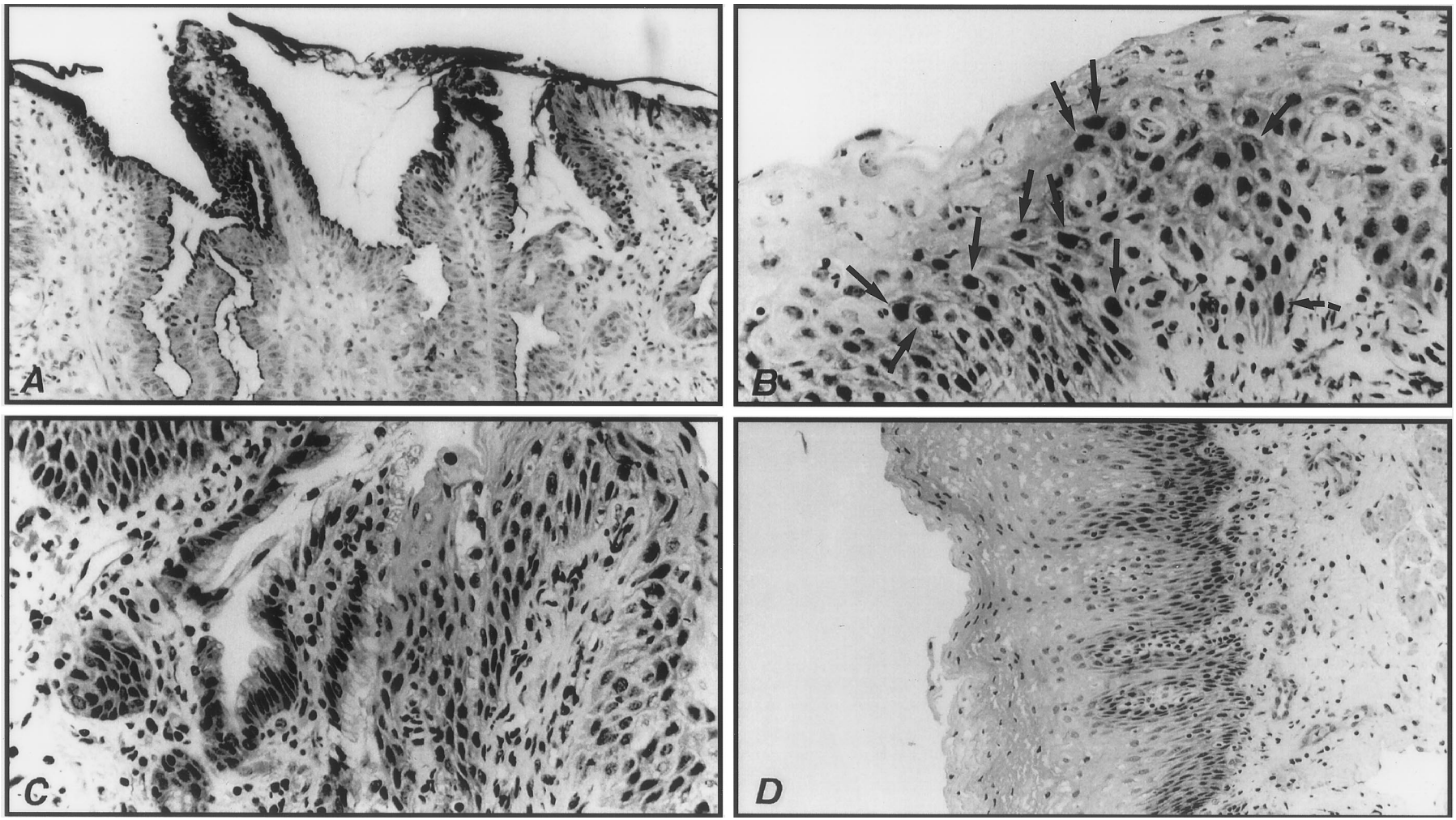

Figure 2. Histopathology of esophageal biopsies. **A**, Biopsy at 12 days of age shows villiform mucosa with surface goblet cells (darkly stained) and moderate acute inflammation (alcian blue/PAS; original magnification, ×50). **B**, Biopsy at 20 months of age from 1–2 cm above lower esophageal sphincter (LES) shows prominent basal hyperplasia with thinned epithelium and neutrophil infiltrate. Arrows denote viral cytopathic effect with numerous inclusions (hematoxylin-eosin; original magnification, ×100). **C**, Biopsy at 20 months of age from 1–2 cm above LES shows changes similar to those seen in **B** in region with residual glandular epithelium (hematoxylin-eosin; original magnification, ×100). **D**, Biopsy after acyclovir treatment at 20.5 months of age shows marked resolution of inflammation seen previously, thicker mucosa, and no evidence of glandular epithelium or viral cytopathic effect (hematoxylin-eosin; original magnification, ×50).

tion. Alternatively, neural damage caused by VZV infection may have resulted in dysautonomia with secondary chronic reflux and esophagitis. Barrett's esophagus and VZV infection of adjacent esophageal mucosa were evident at 12 days of age, supporting the hypothesis that VZV infection of the esophagus led to the development of specialized intestinal metaplasia. At 20 months of age, our patient again had active esophageal VZV infection that most likely represented reactivation of virus, although we cannot discount the possibility of longstanding, persistent VZV esophagitis. Intermittent VZV reactivation could explain the persistence of abnormal esophageal epithelium despite medical and surgical anti-reflux therapy.

In situ hybridization of esophageal tissue obtained from our patient with congenital VZV infection at 12 days and 20 months of age revealed epithelial cells with nuclear VZV DNA and inflammatory cells without VZV. This pattern of infection has been described in skin biopsy specimens obtained from patients with zoster [5, 10, 11]. The presence of VZV in these esophageal biopsy specimens also was confirmed by immunohistochemistry, which detected VZV immediate-early proteins (either IE62 or IE63) at both time points, indicating either active or latent VZV infection. The late VZV gene product, gE, was detected in esophageal biopsy specimens at 20 months before treatment with acyclovir, indicating the presence of replicating virus.

All three VZV proteins studied were found in both the nuclei and cytoplasm of infected cells. The apparent nuclear localization of gE in this study is consistent with the cytoplasmic and nuclear localization of gE in epithelial cells with active infection [10]. IE62 and IE63 have been detected in the cytoplasm of human neurons with latent VZV infection and in the nuclei and cytoplasm of human neurons with reactivated virus [12].

After treatment with acyclovir, there was clinical and histopathologic improvement. We were unable to demonstrate gE in this specimen, although both VZV DNA and IE62 protein were still detectable. Given the absence of gE, this pattern of gene expression is suggestive of latent infection in the esophagus, a novel site for latency. However, because the infant also underwent repeat fundoplication during this course of acyclovir, it is impossible for us to know whether her favorable response and our inability to demonstrate evidence of replicating virus was due to medical or surgical intervention or both.

Using these data, we hypothesize the following sequence of events. Our patient had congenital varicella syndrome associated with specialized intestinal metaplasia of the esophagus, esophageal zoster at 12 days, cutaneous zoster at 5.5 weeks (for which she received 7 days of acyclovir therapy), and either a recurrence or persistence of esophageal zoster at 20 months, with an apparent resolution after acyclovir treatment. Children infected with VZV in utero are at risk of developing zoster during infancy [13]. Although VZV typically reactivates no more than once in a person's lifetime, in some instances, multiple episodes of reactivation occur, such as occurs in patients with HIV infection [14]. The patient described in this report was not infected with HIV but may be predisposed to multiple episodes of zoster because of inadequate cellular immunity resulting from VZV infection early in gestation [15]. Previous experience indicates that immune responses to subsequent episodes of zoster are sufficient to maintain VZV latency in children with congenital varicella syndrome. Whether cessation of VZV replication in this case will have a favorable impact on the patient's esophageal lesions is yet to be determined.

References

1. Pastuszak AL, Levy M, Schick B, et al. Outcome after maternal varicella infection in the first 20 weeks of pregnancy. N Engl J Med **1994**;330:901–5.
2. Savage MO, Moosa A, Gordon RR. Maternal varicella infection as a cause of fetal malformations. Lancet **1973**;1:352–4.
3. Spechler SJ, Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. Gastroenterology **1996**;110:614–21.
4. Lungu O, Annunziato PW, Gershon A, et al. Reactivated and latent varicella-zoster virus in human dorsal root ganglia. Proc Natl Acad Sci USA **1995**;92:10980–4.
5. Annunziato PW, Lungu O, Gershon A, Silvers DN, LaRussa P, Silverstein SJ. In situ hybridization detection of varicella zoster virus in paraffin-embedded skin biopsy specimens. Clin Diagn Virol **1996**;7:69–76.
6. LaRussa P, Steinberg SP, Seeman MD, Gershon AA. Determination of immunity to varicella-zoster virus by means of an intradermal skin test. J Infect Dis **1985**;152:869–75.
7. Gill RA, Gebhard RL, Dozeman RL, Sumner HW. Shingles esophagitis: endoscopic diagnosis in two patients. Gastrointest Endosc **1984**;30:26–7.
8. Kim SL, Waring JP, Spechler SJ, et al. Diagnostic inconsistencies in Barrett's esophagus. Gastroenterology **1994**;107:945–9.
9. Dahms BB, Rothstein FC. Barrett's esophagus in children: a consequence of chronic gastroesophageal reflux. Gastroenterology **1984**;86:318–23.
10. Nikkels AF, Debrus S, Sadzot-Delvaux C, Delvenne P, Rentier B, Pierard GE. Comparative immunohistochemical analysis of herpes simplex and varicella-zoster infections. Virchows Arch A Pathol Anat Histopathol **1993**;422:121–6.
11. Nikkels AF, Delvenne P, Debrus S, et al. Distribution of varicella-zoster virus gpI and gpII and corresponding genome sequences in the skin. J Med Virol **1995**;46:91–6.
12. Lungu O, Panagiotidis CA, Annunziato PW, Gershon AA, Silverstein SJ. Aberrant intracellular localization of varicella-zoster virus regulatory proteins during latency. Proc Natl Acad Sci USA (in press).
13. Brunell PA. Varicella in pregnancy, the fetus, and the newborn: problems in management. J Infect Dis **1992**;166(suppl 1):S42–7.
14. Jura E, Chadwick E, Josephs SH, et al. Varicella-zoster virus infections in children infected with human immunodeficiency virus. Pediatr Infect Dis J **1989**;8:586–90.
15. Terada K, Kawano S, Yoshihiro KY, Morita T. Varicella-zoster virus (VZV) reactivation is related to the low response of VZV-specific immunity after chickenpox in infancy. J Infect Dis **1994**;169:650–2.