Supplemental Table 1. Patients in the study with achalasia

Patient ID #	Gender	Age	BMI	Race	Achalasia subtype	Varicella vaccine	PPI use	Surgery
1	Male	68	33.5	Caucasian	2	No	Yes	Heller Myotomy
2	Female	63	35.5	Caucasian	3	No	Yes	Heller Myotomy
3	Male	55	35.6	Caucasian	3	No	Yes	Heller Myotomy
4	Male	65	19.3	Caucasian	2	No	Yes	Heller Myotomy
5	Female	41	19.9	Caucasian	2	No	Yes	Heller Myotomy
6	Female	75	21.6	Caucasian	1	No	Yes	Heller Myotomy
7	Male	72	20.1	African-American	3	No	Yes	Heller Myotomy
8	Male	71	29.3	Caucasian	2	No	Yes	Heller Myotomy and Esophagectomy
9	Male	48	18.2	Caucasian	2	No	Yes	Heller Myotomy
10	Male	77	26.3	Caucasian	3	No	Yes	Heller Myotomy
11	Male	60	29.3	Caucasian	1	No	Yes	Heller Myotomy
12	Female	70	24.3	Caucasian	2	No	Yes	Heller Myotomy

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13	Female	18	21.2	Caucasian	2	No	Yes	Heller Myotomy
14	Female	81	29.8	Caucasian	1	No	Yes	Heller Myotomy
15	Male	36	28.0	Caucasian	2	No	Yes	Heller Myotomy

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Table 2. VZV gene expression in patients with achalasia undergoing myotomy

Patient	Saliva	DNA	Transcripts	Protein
v1	+	+	+	+
v2	+	+	+	+
v3	+	-	-	-
v5	+	-	+	+
v6	+	-	+	+
v7	-	+	+	+
V8	-	-	+	-
v9	-	+	+	-
v11	+	-	-	-
v12	+	-	+	+
v13	+	-	+	-
v14	+	+	+	+
v15	+	+	+	+
v16	+	+	+	+
v17	+	-	+	+

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Supplementary Methods:

The study was approved by the Vanderbilt University Medical Center and Columbia University's Institutional Review Boards (IRB #191284). All patients provided informed consent.

Collection and processing of specimens

During Heller myotomy, a surgical specimen of the LES muscularis propria was excised and placed on ice, and immediately transported for processing. A portion of the specimen was fixed with 4.0% formaldehyde (from paraformaldehyde) in 0.1 M phosphate buffer and prepared for histological examination. Slides were stained with hematoxylin and eosin (H&E) and reviewed by an expert gastroenterologist pathologist (MKW). The remainder of the tissue was set aside to be processed for the analysis of VZV DNA, transcripts, and protein.

Saliva was collected by passive drool into the tubes of OriGene-Discover kits (Ottawa, Canada) or on swabs placed in the mouth for approximately 3 minutes. Swabs were subsequently soaked in sterile water and stored in buffer (50 mM Tris, pH 8.0, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium azide) at 4°C. DNA was extracted with DNeasy Blood and Tissue Kit (Qiagen, Valencia, California).

PCR analysis and immunocytochemistry

Tissue specimens to be analyzed were divided. One portion, which had been fixed in 0.1 M phosphate-buffered 4% formaldehyde (from paraformaldehyde), was embedded in OCT medium, frozen with liquid N_2 and sectioned in a cryostat-microtome for

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immunocytochemistry. DNA was extracted from the remaining tissue (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA). DNA (100 ng) was subjected to PCR amplification (Eppendorf SmartCycler) in 20 µl of reaction mixture for 35 cycles. The PCR product (1.0 µl) was subjected to further amplification with sets of nested primers for each of the genes analyzed for an additional 32 cycles. Precautions taken to avoid contamination included opening PCR tubes only in a fume hood and analysis of negative control samples simultaneously with experimental samples. Cellular DNA was also examined with primers for human β-globin or glyceraldehyde 3-phosphate dehydrogenase to validate the detection of DNA. Cellular genes were amplified in every experiment. Primers used for PCR and nested PCR were designed to amplify VZV ORFs 29, 40, and 67 (Table1). PCR products were separated on 2% agarose gels, and digital images were obtained for documentation. RNA was extracted with Trizol (Invitrogen, Carlsbad, CA; manufacturer's instructions were followed) from the same tissue from which DNA was extracted. RNA was treated with DNase I (4U/100 µl) to remove possible DNA contamination. Total RNA (3.0 µg) was subjected to reverse transcription with Maloney Murine Leukemia Virus reverse transcriptase (Promega, Madison, WI) in 25 µl of reaction mixture to obtain cDNA. The cDNA (1.0 µl) was subjected to PCR amplification in 20 µl of reaction mixture for each of the genes analyzed. β-actin universal primers were used as controls both for RNA extraction and cDNA preparation.

Sections of fixed frozen tissue were immunostained to identify neuronal cell bodies, neurites, and VZV late antigens in myenteric ganglia. Antibodies to β3-tubulin (Thermo Fisher Invitrogen), PGP9.5 (ubiquitin carboxyl terminal hydrolase 1 [UCHL1]; rabbit monoclonal, Cedarlane Laboratories) and peripherin (rabbit monoclonal, EnCor Biotechnology) were used as

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neural markers. Antibodies to gE (ORF68; monoclonal; Virusys Corporation), gH (ORF37; monoclonal, Virusys Corporation), and ORF40p (mouse monoclonal; Novus Biologicals) were used to detect cells and processes infected with VZV. Sites of primary antibody binding were detected with appropriate species-specific secondary antibodies labeled with contrasting fluorophores (Alexa 488 or 594; Thermo Fisher Invitrogen). After immunostaining, slides were treated with True View™ (Vector Laboratories) for 30-40 sec at room temperature to quench autofluorescence (primarily of elastic fibers). Negative controls, in which primary antibodies were omitted, were processed along with the experimentally analyzed tissues. Nuclei were sometimes counterstained with bisbenzimide (Sigma-Aldrich, St. Louis, MO) to aid the morphological examination of tissue.

Data Collection, Statistics, and Rigor

Patient characteristics and data collected from physiological testing and immunocytochemistry were collected and stored into REDCap (Research Electronic Data Capture). Confounding bias was addressed by collecting relevant information on biological and physiological data. Analysis of the results of the data used the statistical package R (4.0.2, http://www.r-project.org, R Foundation for Statistical Computing and GraphPad Prism version 9 for Macintosh).

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