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Neural Autoantibody Profile of Primary Achalasia

Robert E. Kraichely, M.D.[1], **Gianrico Farrugia, M.D.**[1], **Sean J. Pittock, M.D.**[2,3], **Donald O. Castell, M.D.**[5], and **Vanda A. Lennon, M.D., Ph.D.**[2,3,4]

[1] Enteric NeuroScience Program, Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, MN

[2] Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN

[3] Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN

[4] Department of Immunology, Mayo Clinic College of Medicine, Rochester, MN

[5] Medical University of South Carolina, Charleston, SC

Abstract

The etiology and pathogenesis of primary achalasia are unknown. Postulated mechanisms include autoimmune, viral-immune, and central neurodegenerative. The aim of this study was to investigate the serum profile of neural autoantibodies in patients with primary achalasia. Coded sera from 70 patients with primary achalasia and 161 healthy control subjects, matched in sex, age and smoking habits, were screened for antibodies targeting neuronal, glial and muscle autoantigens. No specific myenteric neuronal antibody was identified. However, the overall prevalence of neural autoantibodies in patients with primary achalasia was significantly higher than in healthy control subjects (25.7% vs 4.4%, $p < 0.0001$). Most noteworthy was the 21.4% frequency of glutamic acid decarboxylase-65 (GAD65) antibody in patients with achalasia (versus 2.5 % in control subjects), in the absence of diabetes or companion antibodies predictive of type 1 diabetes. This profile of autoantibodies suggests an autoimmune basis for a subset of primary achalasia.

Keywords

autoimmune gastrointestinal dysmotility; achalasia; autoimmune; neural autoantibodies; glutamic acid decarboxylase-65

Introduction

Achalasia has been recognized as an esophageal motility disorder for more than three centuries [1]. Its contemporary definition is a characteristic aperistalsis of the esophagus with inadequate lower sphincter relaxation, occurring as a primary (idiopathic) disease or secondary to an infectious or neoplastic disease, myopathy, traumatic or toxic nerve injury, or metabolic/infiltrative disease [2,3]. The pathology of primary achalasia is well established. Typically, inhibitory nitrergic myenteric plexus neurons are lost, and degenerating neurons and ganglia are often surrounded by lymphocytes and eosinophils [4]. Excitatory cholinergic innervation initially is relatively spared [5]. The cause of neuronal degeneration in primary achalasia is not known.

Reprint requests and correspondence: Vanda A. Lennon, M.D., Ph.D., Neuroimmunology Laboratory, 828 Guggenheim Building, Mayo Clinic, Rochester, MN 55905; lennon.vanda@mayo.edu; Tel: (507)-284-7514; Fax: (507)-284-1814.

An association with class 2 major histocompatibility complex haplotypes (HLA-DQ and HLA-DR) supports an autoimmune mechanism for primary achalasia [6], as do immunohistochemical demonstrations of cytotoxic T lymphocytes in the esophageal wall (CD3+/CD8+, many containing granzyme B) [7]. In a study of 92 patients with primary achalasia, Ruiz-de-Leon et al reported finding non-organ-specific autoantibodies in more than 50% of patients [8]. Reports that some patients' antibodies bind to myenteric neurons [9,10]. elicited interest in the potential role of neural-restricted autoimmunity as a pathophysiological effector of achalasia. Neuron-specific autoimmunity targeting nuclear Hu proteins or the cytoplasmic collapsin response-mediator protein-5 (CRMP-5) [9–13], and profiles of neural autoantibodies, predominantly directed at plasma membrane cation channels [14–16], have been documented in both paraneoplastic and idiopathic forms of gastrointestinal dysmotility affecting various levels of the gastrointestinal (GI) tract. The present study provides a comprehensive evaluation of the frequency and specificity of serum autoantibodies directed against neuronal, glial and muscle antigens in patients ascertained clinically by presentation with an idiopathic anatomically limited GI dysmotility, primary achalasia.

Methods

Patients

We collected serum between 1996 and 2005 from patients in whom a clinical diagnosis of primary achalasia based on esophageal manometry and no evidence of a secondary cause of achalasia on an imaging study and on endoscopic evaluation of the esophagogastric junction was made at Mayo Clinic Rochester (MN) or Drexel University (PA). Absolute inclusion criteria were aperistalsis with incomplete lower esophageal sphincter relaxation, and negative imaging for local cancer or infiltrative disease. Control sera were collected in 2005 from 161 healthy age-and sex-matched residents of Olmsted County. Institutional Review Board approval for the study was obtained at both Mayo Clinic Rochester and Drexel University. All achalasia patients completed medical history questionnaires (including diagnoses of diabetes, thyroid disease, pernicious anemia, vitiligo, rheumatoid arthritis or systemic lupus erythematosus), smoking and environmental exposures with known risk for cancer (tobacco smoke or asbestos) and family history of achalasia, cancer or autoimmunity.

Serological Analyses

All sera were tested blinded to clinical diagnoses.

Neural autoantibodies—(a) Radioimmunoprecipitation assays were used to test for autoantibodies to nicotinic acetylcholine receptors (AChR; both ganglionic-type [α3 subunit-containing] and muscle-type), neuronal voltage-gated potassium channels (α-dendrotoxin-sensitive) and Ca^{2+} channels (P/Q-type and N-type) and glutamic acid decarboxylase-65 (GAD65) [14-17]; (b) ELISA for skeletal muscle striational (cytoplasmic) antibodies [17]; (c) indirect immunofluorescence for neuronal nuclear and cytoplasmic autoantibodies (including anti-neuronal nuclear autoantibody-1 [ANNA-1; also known as anti-Hu], CRMP-5-IgG and GAD65)[13,18,19]and (d) western blot (recombinant human protein) for CRMP-5-IgG [13].

Other organ-specific autoantibodies—Additional markers of susceptibility to type 1 diabetes included islet cell tyrosine phosphatase-like protein (IA-2) and insulin (radioimmunoprecipitation assays using ^{125}I-labelled recombinant human antigens). Other markers of organ-specific autoimmunity included gastric parietal cell antibody (GPC; indirect immunofluorescence assay), and thyroid cytoplasmic antibodies (thyroglobulin and microsomal/thyroperoxidase; latex agglutination) [19].

To minimize interference by non-organ-specific autoantibodies (anti-nuclear antibody [ANA], smooth muscle [SMA] and anti-mitochondrial [AMA]) in immunofluorescence assays, we pre-absorbed all sera prior to testing (three times with liver powder, at 1:240 dilution). The substrate was a composite of frozen mouse tissues (stomach, kidney, cerebellum and midbrain), 4 μ sections, post-fixed for 10 minutes in 10% formalin. Neural-specific binding of a standardized fluorescein-conjugated anti-human-IgG was scored positive or negative using an Olympus BX51 fluorescence microscope, equipped with fluorescein-optimized illuminators and filters, U Plan Fluorite 10X (NA 0.3, WD 10 mm) objective and Widefield 10X eyepiece. Further absorptions, and judicious titration in doubling dilutions, were performed as needed. Endpoint dilutions were recorded for positive results.

Results

Demographics

The study group comprised 70 patients with a diagnosis of primary achalasia (57% female); the control group comprised 161 healthy subjects (54% female). No patient had esophageal cancer or thymoma. All study subjects were adult except for a single patient aged 15 yrs. The median ages of the achalasia group and the control group were 52.5 years and 53.5 years (ranges 15–87 and 18–83). Thirty percent of the achalasia patients and 46% of the control subjects had a positive smoking history. Ten of the achalasia patients had documented thyroid disease (14%), three had diabetes (4%), four had known asbestos exposure, two had neuropathy and pone had vitiligo. No patient had a diagnosis of pernicious anemia or a family history of achalasia.

Autoantibodies Detected

No achalasia patient or healthy control subject had a neuronal nuclear (ANNA-1) or cytoplasmic (CRMP-5-IgG) or ganglionic ACh receptor autoantibody, all of which have been reported in the context of paraneoplastic achalasia [11,13,14,16]. However, 18 (25.7%) of the patients with primary achalasia had one or more neural autoantibodies: skeletal muscle AChR or striational, neuronal voltage-gated cation channel (potassium channel or N-type calcium channel) or GAD65. By contrast, only 4.4% of healthy subjects had one or more of these autoantibodies. Remarkably, all the detected neural autoantibodies are frequent accompaniments of thymoma [20], but no patient had evidence of thymoma. It is further remarkable that 21% of the achalasia patients had GAD65 antibody (compared to 2.5% in controls; $p < 0.0001$, Fisher's exact test), but only one had clinically documented diabetes mellitus and none had coexisting pancreatic islet cell antibodies (IA-2 or insulin) which are of additional serological predictive value for susceptibility to type 1 diabetes [21]. Our data suggest that neural autoimmunity may underlie more than one in four cases of achalasia. This suggestion is supported by our additional finding that 41% of the achalasia patients in total had at least one other organ-specific autoantibody (thyroid [19% vs 13%] or GPC antibodies [5.7% vs 1.9%]) compared to 18% of healthy controls ($p < 0.0004$, Student's *t*-test).

Discussion

This study documents a remarkably high prevalence of neural antibodies in patients with primary achalasia. No single autoantibody emerged as consistently positive in the study population but, curiously, we detected antibodies that are frequently encountered with thymoma [20]. Paraneoplastic achalasia resembles primary achalasia functionally and pathologically. Loss of ganglionic neurons is characteristic of both disorders. The lack of ANNA-1 and CRMP-5 autoantibodies and a mean interval of 5.7 years from diagnosis to collection of sera minimized the likelihood that achalasia in the patients of this report had a paraneoplastic basis [11,13]. It is noteworthy that we did not detect any myenteric neuronal-

specific autoantibody. Our screening protocol for detecting enteric neuronal autoantibodies minimized interference by non-neuron-specific antibodies by extensively pre-absorbing each serum with liver powder and by using more diluted serum (1:240) and more sensitive fluorescence microscopy than are customarily used in serological studies. Nevertheless, we incidentally noted non-organ-specific autoantibodies (ANA, AMA or SMA) in six patients (8.6%). Anti-nuclear and anti-mitochondrial antibodies bind more avidly to myenteric plexus neurons than to mucosal or smooth muscle cells of the gut (V.A. Lennon, personal observation). We suspect that the 39% to 50% frequency of "myenteric neuronal antibodies" reported in patients with idiopathic achalasia by other investigators[8,22] is attributable to non-organ-specific autoantibodies. The fact that we used stomach rather than esophagus as gut substrate is an unlikely explanation for non-detection of IgG binding to enteric neurons. Myenteric neurons throughout the gastrointestinal tract, from esophagus to rectum, are known to be susceptible to autoimmune attack in a paraneoplastic context [11].

The striking frequency of GAD65 autoantibody in our cohort of patients with achalasia (11-fold higher than in controls, $p < 0.0001$) is of particular note. The enzyme GAD65 converts glutamic acid to gamma amino butyric acid, and is expressed in GABAergic nerve terminals in the enteric nervous system[23] as well as in pancreatic β islet cells and the central nervous system. GAD65 antibodies are found in 80% of patients with type 1 diabetes mellitus (usually in low titer) and in approximately 20% of patients with various organ-specific neurological disorders, including myasthenia gravis, Lambert-Eaton syndrome, autoimmune dysautonomias and encephalopathies [19,24]. In the present study, only one GAD65 antibody-positive patient with primary achalasia was diabetic, and none had IA-2 or insulin autoantibodies which complement GAD65 antibody as a serological predictor of type 1 diabetes[21].

In summary, the profile of marker autoantibodies we report from this study of patients with primary achalasia suggests that a significant proportion of cases may have an organ-specific autoimmune basis. This finding is in line with the detection of lymphocytic infiltrates in the myenteric plexus, predominantly T cells, in mild or clinically early achalasia. The positive CD8 and TIA-1 phenotypes of these T-lymphocytes suggest that cytotoxic T cells specific for esophageal neuronal peptides (possibly derived from GAD65) may be the principal effectors of myenteric neuron destruction [7]. Our findings do not define a specific pathogenic autoantibody, nor do they explain why the insult to enteric neurons is limited to the esophagus. Most cases of achalasia are isolated, but achalasia may coexist with dysmotility at multiple levels of the GI tract [14] and it is sometimes associated with biliary dyskinesia [25]. Organ-specific autoantibody profiles were the first clue that myasthenia gravis and Lambert-Eaton syndrome had an organ-specific autoimmune basis [26,27]. The autoantigens causally pertinent to impairment of neuromuscular transmission in those two disorders proved to be cation channels in the synaptic plasma membranes of muscle (AChR) and the motor axon terminal (voltage-gated P/Q-type calcium channel) [28,29]. Potentially pathogenic neural autoantibodies, and corresponding plasma membrane target antigens, remain to be identified in patients with achalasia.

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Table

Autoantibody Frequency (%) and Range of Values in Patients and Healthy Controls

Autoantibody detected	Achalasia (n=70)	Range of values (nmol/L or titer)	Controls (n=161)	Normal values (nmol/L or titer)
Neural-specific				
Nicotinic acetylcholine receptor:				
ganglionic	0	---	0	0.00–0.02
muscle	1.4	0.04	0	0.00–0.02
Neuronal calcium channel:				
N-type	1.4	0.08	0	0.00–0.03
P/Q type	0	---	<1	0.00–0.02
Voltage-gated potassium channel	1.4	0.04	<1	0.00–0.02
Anti-neuronal nuclear autoantibody, type 1	0	---	0	negative
CRMP-5	0	---	0	negative
GAD65*	21.4	0.03–4.55	2.5	0.00–0.02
Striational	2.9	240–1920	<1	<60
1 or more	25.7	--	4.4	--
Other organ-specific				
Gastric parietal cell	5.7	positive	1.9	negative
IA-2	1.4	0.03	n.t.	0.00–0.02
Insulin	0	---	3.7	0.00–0.02
Thyroid	20.0	100–6400	12.4	<100

* GAD65 antibody, but no other autoantibody, was significantly more frequent in patients with primary achalasia than in healthy control subjects ($p < 0.0001$, Fisher's exact test).