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TRYPTOPHAN HYDROXYLASE AUTOANTIBODIES AS MARKERS OF A DISTINCT AUTOIMMUNE GASTROINTESTINAL COMPONENT OF AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE 1

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Abbreviations: AIRE, Autoimmune regulator; APS-1, autoimmune-polyendocrine-syn- drome type 1; EC, enterochromaffin cells; EE, enteroendocrine cells; GI, gastrointestinal; GID, gastrointestinal dysfunction; 5-HT, serotonin; IEL, intraepithelial lymphocytes; TPHAbs, tryptophan hydroxylase.

Abstract

Background: Autoantibodies to tryptophan hydroxylase (TPHAbs) directed against serotonin-produc- ing enterochromaffin cells (EC) have been reported in autoimmune-polyendocrine-syndrome type 1 (APS-1) patients with

gastrointestinal dysfunction (GID). Serotonin plays a critical role in enteric function and its peripheral blood levels reflect serotonin release from the gastrointestinal tract.

Aims: We test the hypothesis that TPHAbs mark a distinct autoimmune component of APS-1 char- acterized by an autoimmune attack toward EC, which results in clinical GID.

Methods: TPHAbs were measured in 64 APS-1 patients. Endoscopy with gastric (antrum/body) and duodenal biopsy was carried in 16 TPHAbs+ patients (8 with and 8 without GID) and in 2 TPHAbs- patients (without GID). Immunohistochemistry of biopsy specimens was carried out using antibodies to serotonin, chromogranin-A, CD3, CD4, CD8, and CD20. Serotonin serum levels were measured in TPHAbs+ and TPHAbs- patients who had endoscopy.

Results: Thirty-seven of 64 patients were TPHAbs+ (11/12 with GID and 26/52 without GID; P < .001). Gastric and duodenal biopsies in all 8 TPHAb+ patients with GID showed lymphocytic infil- tration with increased CD3+CD8+ intraepithelial lymphocytes and absence of EC. Furthermore, mean serotonin serum levels were below the normal range in TPHAb+ patients with GID (P < .01). In 8 TPHAb+ patients without GID gastric and duodenal biopsies showed different grades of inflammatory infiltration and reduced number of EC. Mean serotonin serum levels were near the lower limit of the normal range. In all TPHAbs+ patients the biopsies showed a reduced number of chromogranin-A positive cells consistent with enteroendocrine cells depletion. TPHAbs- pa- tients without GID showed normal gastrointestinal mucosa and serotonin serum levels.

Conclusions: TPHAbs appear to be markers of a distinct autoimmune component of APS-1. Progressive involvement of the gastrointestinal EC leads to the transition from preclinical to clinical disease, character- ized by GID and reduced serotonin serum levels.

Introduction

utoimmune polyendocrine syndrome type 1 (APS-1) is a rare autosomal-recessive disorder caused by mu- tations in the autoimmune regulator (AIRE) gene (1, 2). APS-1 serves as a model of autoimmune disease in partic- ular for understanding the relationship between the T-cell tolerance and the immune response against the self (3). The clinical phenotype is characterized by 3 main disease components (chronic mucocutaneous candidiasis, chronic hypoparathyroidism, and Addison's disease) often pre- senting together with other autoimmune diseases early in childhood and is associated with the presence of serum autoantibodies to different self-antigens (4 – 6).

Patients with APS-1 often present with gastrointestinal (GI) dysfunction (GID) that can be autoimmune (gastritis, hepatitis, idiopathic cholecystokinin deficiency, celiac dis- ease) or nonautoimmune (cystic fibrosis, pancreatic insuf- ficiency, lymphangectasia, and intestinal infections with Candida albicans or Giardia lamblia) or sometimes is classed as idiopathic (4 – 8). GI symptoms, such as recur- rent diarrhea and steatorrhea or periodic or chronic con- stipation, can lead to malabsorption and cause reduction of the effectiveness of the replacement therapy in patients with deficiency of endocrine function (6).

It has been suggested that an autoimmune response di- rected to the enteroendocrine cells (EE) may be responsible for GIDin a subset of patients with idiopathic GID(9 –13). The EE cells represent the enteric endocrine system, se- creting various GI peptides; these cells are scattered throughout the GI tract and are generally identified by chromogranin-A positivity by immunostaining on the tis- sue specimens. There are at least 15 subtypes of EE, se- creting different peptide hormones that elaborately con- trol physiological and homeostatic functions in the digestive tract, particularly postprandial secretion and motility. A set of biogenic amines are also synthesized and secreted, particularly serotonin (5-HT) from the diffusely distributed enterochromaffin cell (EC) (14).

It has been found that autoantibodies to tryptophan hydroxylase (TPHAbs) have been associated with the id- iopathic form of GID in patients with APS-1 (9, 10).

Tryptophan hydroxylase is the rate-limiting enzyme in the synthesis of 5-HT and is expressed mainly in the EC of the gut. The release of 5-HT from EC initiates the activa- tion of motor, secretory, and vasodilatory reflexes in re- sponse to luminal stimuli in the gut, and serotonin levels in the peripheral blood are thought to reflect serotonin release from the GI tract (15–20). To date the intestinal biopsies have been analyzed in only a few APS-1 patients with GID who were positive for TPHAbs and showed the absence of serotonin-containing EC but without the evi- dence of lymphocytic infiltration in the duodenal mucosa (9, 11, 21).

To test the hypothesis of an autoimmune attack toward EC further, we analyzed TPHAbs in 64 Italian patients with APS-1 and in 16 TPHAbs+ patients we measured serotonin plasma levels and examined GI biopsies using standard histological and immunohistochemical techniques.

Materials and Methods

Patients

We investigated 64 Italian patients with APS-1 (42 female and 22 male patients; mean age 33.7 y; range 7–76 y). Idiopathic GID was diagnosed in patients with recurrent chronic diarrhea with malabsorption and weight loss without any significant allergic, immunologic, infectious, and metabolic cause, or chronic con- stipation. Constipation and diarrhea periods were determined using patients records (in case of hospitalization) or obtained by interviewing the patients or their endocrinologist/pediatrician. According to stool frequency and/or consistency, defecation less than 3 times per week or hard, lumpy stool was defined as con- stipation, whereas defecation more than 3 times a day or soft, watery stool was defined as diarrhea.

Exocrine pancreatitis was ruled out because of the absence clinical signs or symptoms (no obstructive jaundice or abdominal pain) and normal serum amylase and lipase.

Twelve of 64 patients had idiopathic GID (18.8%; 5 male and 7 female patients; mean age 22.7 y; range 9 –32 y) with mean onset at 10.6 years of age.

A gastroduodenal endoscopy including biopsies of gastric (antrum and body) and duodenal mucosa was carried out in 8 TPHAbs+ patients with GID (when symptomatic) and in 8 TPHAbs+ patients without GID. In addition, colonic biopsies were available for 3 patients (2 with and 1 without GID; Table 3, patients 4, 7, 15). The investigation was offered to all TPHAbs+ patients and to 10 TPHAbs- patients (with or with- out GID): patients' personal agreement was the only criteria of selection.

Six archival samples of normal gastric and duodenal mucosa and samples obtained from 2 TPHAbs- patients with APS-1 were used for reference.

Written informed consent was obtained from all patients and the study was approved by the local ethic committee.

TPHAb assays

TPHAbs were detected in serum samples by immunoprecipita- tion assay with 35S-labeled full-length TPH, as described previously (10), and the upper normal limit of the antibody index was 2.3. Sera from 60 healthy blood donors were analyzed as controls.

Histology and immunohistochemistry

Biopsies were formalin-fixed and paraffin-embedded and sec- tions were stained with hematoxylin and eosin and the modified Giemsa stain (for gastric biopsies alone) to identify Helicobacter pylori infection (22). The modified Sydney System was used to grade and stage the gastric biopsies (22). In addition, duodenal sections were assessed for villus-crypt ratio, lamina propria in- flammation (quantified as normal or increased), and the presence of intraepithelial lymphocytes (IELs) (23).

Immunohistochemical tests were carried out by standard techniques using commercially available antibodies to serotonin (Novocastra, Newcastle, United Kingdom), chromogranin-A (Dako, Glostrup, Denmark), CD3 and CD4 (Novocastra), and CD8 and CD20 (Dako), according to the manufacturers' protocols.

Quantitative chromogranin-A and serotonin immunohisto- chemistry results were obtained by counting the number of pos- itive cells in an area of mucosa bounded by 50 crypts (the given number of positive cells is the mean of positive cells counted in at least 3 different microscopic fields for each evaluation). Six archival samples of normal gastric and duodenal mucosa and samples obtained from 2 TPHAbs- patients with APS-1 without GID were used to establish a normal mean and SD for our study. According to previous studies (24, 25), a count value 2 SDs below the mean of controls was defined as marked reduction of EE or EC cells. One SD below the mean was considered borderline reduction. The pathologist read the samples and was blinded to the status of the patient regarding GID and the presence of TPHAbs.

Serotonin serum levels

Patients and controls were tested in the morning after an overnight fast. Serotonin-rich foods, such as walnut, banana, aubergine, tomato, hazelnut, peanut, and avocado, were stopped 72 hours before the study. Drugs that modify GI function or the 5-HT system (analgesics, tranquilizers, or antidepressants) and cigarette smoking were forbidden 48 hours before the study, and alcohol and caffeine were ceased 24 hours before the study. All venous blood samples (2– 4 mL) were drawn into plastic vacuum tubes. Serum was separated by centrifugation of the blood sam- ple at 3000g for 15 minutes at room temperature and stored at

-20°C until assayed.

Serotonin serum levels were measured in the 16 TPHAbs+ patients who agreed to undergo endoscopy using liquid chro- matography and electrochemical detection (26). The reproduc- ibility of all the tests was satisfactory, the coefficient of analytical variation (interassay CV) being <7% for all concentrations of control materials. The normal range was 0.28 – 1.7 μmol/L, ob- tained from 40 healthy controls samples. As controls were also tested, 10 APS-1 patients were negative for TPHAbs and without GID.

Genetic study

All 14 exons of the AIRE gene were analyzed for mutations in the patients with APS-1, as described previously (27).

Statistical analysis

Fisher's exact test and Wilcoxon test were used for statistical evaluation in APS-1 patients.

Results

Patients

The clinical manifestations of APS-1 and the prevalence of disease components in the 64 APS-1 patients and in the 16 TPHAbs+ patients who consented to have an endo- scopic study are shown in Table 1.

Diagnosis of each disease was based on typical clinical, immunologic, and biochemical grounds. Each APS-1 pa- tient presented at least 2 of the 3 main diseases (chronic mucocutaneous candidiasis, chronic hypoparathyroid- ism, and Addison disease) (6). Furthermore, we tested 51 of 64 APS-1 patients for interferon-w and 47 (92.2%) were positive. Three of the 4 negative patients carry an AIRE gene mutation in homozygosis or compound heterozygo- sis. The only patient interferon-w-negative and without any detectable AIRE gene mutation was a 36-year-old woman affected by autoimmune Addison's disease (pos- itive for 21-hydroxylase autoantibodies) and chronic mu- cocutaneous candidiasis; she is also TPHAbs- without GI symptoms.

All the 16 TPHAbs+ and 2 TPHAbs- APS-1 patients studied by endoscopy were checked for transglutaminase antibodies and all were negative. Of the other 46 patients who did not have endoscopy, 26 were screened and 1 case (TPHAbs-) was found positive for transglutaminase antibodies.

TPHAbs

Thirty-seven (57.8%) of the 64 patients with APS-1 were positive for TPHAbs. TPHAbs positivity was signif- icantly associated with GID: 11/12 (91.7%) patients with GID were positive compared to 26/52 (50%) without GID ($P < .001$). One of 12 patients with GID was TPHAbs-. The mean TPHAbs index for all positive patients was 102.8 ± 58.1 (mean ± SD). The mean TPHAbs index for patients with GID was 140.8 ± 50.0 (mean ± SD), whereas for patients without GID it was 89.7 ± 55.6 (mean ± SD) ($P = .03$). TPHAbs were not detected in any of the controls.

Histology of biopsy specimens

Results of histologic examination of the biopsy speci- mens are shown in Table 2.

A mild to moderate lymphoplasmacytic infiltration was found in the gastric biopsies of all TPHAbs+ patients. Inflammatory infiltration was characterized by a mainly CD3+ T-cell lymphocytic infiltrate in both gastric antrum (12/16 patients) and body (11/16 patients) with a variable CD4/CD8 ratio (4/1 to 1/19). CD3+/CD8+ T cells were predominant in the intraepithelial infiltrate (Figure 1A). Scattered CD20+ (B cells) were also found, frequently arranged in clusters (5/16 patients) (not shown).

None of the patients had histologic evidence of pyloric metaplasia, pancreatic acinar cell metaplasia, or entero-chromaffin-like cell hyperplasia. A mild active inflammation was observed in the antrum of the H pylori–positive patient (Table 3, patient 16).

Duodenal biopsies were available for 15 TPHAbs+ pa- tients and showed a mild lymphocytic infiltration predom- inantly with CD3+ T cells in 6 of 7 patients with GID (85.7%) and in 4 of 8 patients without GID (50%). In all 10 specimens that showed the signs of lymphocytic infil- trations, an increased population of CD3+CD8+ IELs was also found (Figure 1B).

No other significant histologic changes or enterocyte damage was found in patients who had a duodenal biopsy. The available colonic biopsies showed a mild stromal lym- phocyte infiltration with mildly fibrotic features in 2 patients with GID (Table 3, patients 4 and 7), which was unremark- able in the other patient without GID (Table 3, patient 15). The 2 TPHAbs- APS-1 patients without GID had a normal gastric and duodenal mucosa, which was comparable with that from the 6 controls samples.

Immunohistochemistry of biopsy specimens and serotonin plasma levels

TPHAbs+ patients with GID

The gastric biopsies in all 8 TPHAbs+ patients with GID showed complete absence of serotonin containing EC (Fig- ure 1, D and F). Five of the 7 duodenal biopsies were also negative for EC (Figure 1H) and the other 2 duodenal biop- sies showed only a few serotonin-positive cells. The 2 colonic biopsies were also negative for EC (not shown). Results of immunohistochemistry for chromogranin-A on antral, body, and duodenal biopsies are summarized in Table 3.

The mean serotonin plasma level was 0.16 ± 0.14 μmol/L (Table 3 and Figure 2) and was significantly lower ($P < .01$) compared to that found in TPHAbs- controls (1.01 ± 0.65 μmol/L; range 0.33–2.1 μmol/L) and to the reference normal range.

TPHAbs+ patients without GID

The antrum biopsies were negative for serotonin by immunohistochemistry in 7 of 8 TPHAbs+ patients with-out GID, whereas only a few serotonin-producing EC were found in 1 of 8 patients. In the case of gastric body biopsies, there was no detectable reactivity for serotonin in 6 of 8 patients; 1 patient had only a small number sero- tonin-positive cells and 1 patient had a normal number of serotonin-positive cells. Duodenal biopsies showed that 2 patients had a normal number of serotonin-positive cells, and 5 patients had lower than normal number, whereas in the case of 1 patient serotonin-positive cells were absent (Table 3, patient 10). Only a few serotonin-positive EC were found on 1 available colonic biopsy. Results of im munostaining for chromogranin-A on gastric and duode- nal biopsies are summarized in Table 3.

The mean serotonin plasma level was 0.58 ± 0.41 μmol/L (Table 3 and Figure 2), which was higher than that in TPHAbs+ with the GID group, lower than the control group (1.01 ± 0.65 μmol/L; range 0.33–2.1 μmol/L) and within the reference normal range.

TPHAbs- patients

The 2 TPHAbs- APS-1 patients without GID had an immunohistochemical reactivity for serotonin and chor- mogranin-A comparable with that from the 6 archival samples. Also the serum serotonin levels were within the normal range (Table 3).

Genetic study

Analysis of the AIRE gene revealed the presence of mu tations in all the 16 patients who underwent endoscopy (Table 3).

Discussion

In our study of a large group of Italian APS-1 patients, TPHAbs were found in 57.8% of patients. Furthermore, 11 of 12 patients (91.7%) with idiopathic GID were TPHAbs+. These observations are consistent with previ- ous reports based on other groups of patients (9, 10, 28). In addition, our study provides an interesting insight into the relationship between TPHAbs positivity and autoim- mune processes affecting the gut mucosa. Evidence of a lymphoplasmacytic infiltration of lamina propria has been found in the GI tract of TPHAbs+ patients, charac- terized by an increased population of CD3+CD8+ IELs. A gastric infiltration was apparent in all the TPHAbs+ patients studied being present in the gastric antrum and the body (interestingly, this pangastric distribution differs from the classic type A and type B gastritis recognized in the updated Sydney System) (22). Furthermore, in the pa- tients with clinical GID, the inflammatory infiltration ex- tended to the duodenal and colonic mucosa and the in- tensity of the infiltration was greater in patients with clinical GID compared to TPHAbs+ patients without GID. The observed extensive lymphocytic infiltration in TPHAbs+ patients correlated with a marked reduction in number of EC or with the complete loss of EC throughout the GI tract. The inflammatory infiltration and loss of EC were more extensive in patients with clinical GID who, in addition to the gastric involvement, also showed reduc- tion or absence of serotonin immunoreactivity in the du- odenal and the colonic biopsy specimens. In contrast, the inflammatory infiltration and loss of EC were less exten- sive and prevalently localized in the gastric mucosa of the asymptomatic TPHAbs+ patients. The observed changes affecting EC in TPHAbs+ patients were consistent with reduced immunostaining for chromogranin-A.

Serotonin levels in the peripheral blood are thought to reflect serotonin release from the GI tract (15–20) and in this study we observed that TPHAbs+ patients with GID had mean plasma serotonin levels significantly lower than the normal range (consistent with the loss of EC through- out the GI tract), whereas the TPHAbs+ patients without GID had mean plasma serotonin levels near the lower limit of the normal range (consistent with the incomplete loss of serotonin immunoreactivity in the duodenal and colonic biopsies). This may reflect the autoimmunity related to serotonin-producing EC failure.

In view of the Witebsky criteria (revised by Rose) (29,30) for defining an autoimmune disease, positivity for TPHAbs in APS-1 seems to indicate a distinct GI autoim- mune disease targeting gut EC characterized by the fol- lowing: 1) genetic susceptibility (AIRE gene mutations); 2) lymphocytic infiltration with presence of IELs in the GI tract; and 3) absence of the EC in the gut associated with low plasma serotonin levels.

The natural history of an autoimmune disease often is characterized by progressive phases, usually identified as potential, subclinical, and clinical (7, 31). Similar progres- sive phases were observed in patients in our study. In par- ticular,

patients with a clinical form (Table 3, patients 1– 8) were characterized by genetic predisposition, pres- ence of TPHAbs, clinical GID, diffuse lymphoplasmacytic infiltration in the whole GI tract, absence of EC through- out the GI tract, and low plasma serotonin levels. Patients with a subclinical/preclinical form (Table 3, patients 9 –16) were characterized by the presence of TPHAbs, ab- sence of clinical manifestations, variable inflammatory in- filtration and EC involvement along the GI tract, and plasma serotonin levels in the lower range of normal val- ues. The potential disease, theoretically, would be char- acterized by the presence of TPHAbs in the absence of any impairment of the target organ.

It is not yet known whether changes in serotonin sig- naling contribute to the alterations in GI function, but several lines of evidence support the concept that altered serotonin signaling can lead to changes in gut function. Studies on diarrhea-predominant irritable bowel syn- drome and constipation-predominant irritable bowel syn- drome report changes in 1 or more aspects of serotonin signaling in both senses, increased or decreased function (16, 19, 32, 33). Also on inflammatory bowel disease al- tered serotonin physiology is reported but without univo- cal results (32, 34 –36). Due to the complex interactions (15) between substances released by enteroendocrine cells, cytokines released by local and remote immunologically reactive cells, the activity of the enteric nervous system, the peripherally released peptides and hormones, and the se- rotoninergic signaling, it would be very difficult to predict the effect of the complete or partial loss of EC on GI func- tion, and whether this loss would have a stimulatory or an inhibitory effect. All these components may influence gut physiology. Probably also individual genetic and epige- netic factors (ie, serotonin receptors or serotonin-selective reuptake transporter expressions) contribute to determine the clinical phenotype (37, 38). Further studies are needed to explain these mechanisms and clarify why in APS-1 patients the GID is reported as both diarrhea and constipation.

Treatment options for TPHAbs+ patients with GID present an interesting challenge. As the changes in sero- toninergic signaling are believed to stem from an autoim- mune attack, immunosuppression may well be the most appropriate choice of treatment, as suggested previously (39, 40). However, autoimmune diseases in APS-1 are mostly related to T-cell dysregulation and, although the autoantibodies do not appear to have a directly patho- genic role, they are important hallmarks of the disease and usually have value for diagnosis and prediction of the dis- ease. However, recently the importance of the B cells' in- volvement has been highlighted in the induction of organ- specific autoimmunity in the mouse model of APS-1 (41) and in the human pulmonary component of APS-1 (42): a good response to Rituximab immunotherapy in both sit- uations raises the possibility of proposing similar treat- ment options also in TPHAbs+ patients with severe GID. Screening for TPHAbs could identify a subset of APS-1 patients with, or that can potentially develop if asymp- tomatic, GID of autoimmune etiology. The positivity needs to be confirmed by a subsequent serum

serotonin evaluation and coherent histologic and immunohisto- chemical findings on GI endoscopy to define the disease's stage and to rule out other autoimmune or nonautoim- mune GI diseases. In the case of an absence/marked de- pletion of EC, an immunosuppressive therapy could be considered in addition to standard supportive care. This strategy would lead to early diagnosis and timely treat- ment of patients with GID because of an autoimmune reaction to EC. Serum serotonin level by itself is not spe- cific because it can be altered in other conditions (16, 19, 32–36) and it could be useful to measure it to evaluate the treatment's response without performing endoscopy, or when it cannot be performed.

Finally, TPHAbs positivity could alert the clinician to APS-1 in patients who present with GID symptoms in the absence of other components of the syndrome.

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Table 1. Clinical Manifestation in the 64 APS-1 Patients and in the 16 TPHAbs+ Patients at Inclusion

	Manifestations	Prevalence (%) in 64 APS-1 Patients	Prevalence (%) in 16 Patients Investigated by Endoscopy
Main components	Addison's disease	53 (82.8)	16 (100.0)
	Chronic hypopathyroidism	61 (95.3)	16 (100.0)
	Chronic candidiasis	55 (85.9)	14 (87.5)
Other components	Ectodermal manifestation (ocular, skin and nail manifestations)	28 (43.8)	6 (37.5)
	Autoimmune thyroid diseases	21 (32.8)	6 (37.5)
	Hypergonadotropic hypogonadism	19 (29.7)	6 (37.5)
	Autoimmune gastritis	23 (35.9)	6 (37.5)
	Pernicious anemia	13 (20.3)	7 (43.7)
	Autoimmune hepatitis	14 (21.9)	9 (56.2)
	Gastrointestinal dysfunction	12 (18.8)	8 (50.0)
	Alopecia	20 (31.3)	7 (43.7)
	Vitiligo	14 (21.9)	7 (43.7)
	Diabetes mellitus (type 1)	4 (6.3)	1 (6.2)
	Pituitary failure	5 (7.8)	1 (6.2)

Table 2. Histological Findings in TPHAbs+ Patients

Site	Histological Feature	Prevalence (%) in TPHAbs+	TPHAbs+ With GID	TPHAbs+ Without GID
Antrum		16 cases	8 cases	8 cases
	Lymphoplasmacytic infiltrate	16/16 (100)	8/8 (100)	8/8 (100)
	Increased IELs	13/16 (81.3)	8/8 (100)	5/8 (62.5)
	Atrophy	0/16	0/8	0/8
	Intestinal metaplasia	1/16 (6.3)	0/8	1/8 (12.5)
	Regenerative hyperplasia	6/16 (37.5)	3/8 (37.5)	3/8 (37.5)
Gastric body		16 cases	8 cases	8 cases
	Lymphoplasmacytic infiltrate	16/16 (100)	8/8 (100)	8/8 (100)
	Increased IELs	12/16 (75.0)	8/8 (100)	4/8 (50.0)
	Atrophy	6/16 (37.5)	3/8 (37.5)	3/8 (37.5)
	Intestinal metaplasia	3/16 (18.8)	1/8 (12.5)	2/8 (25.0)
	Regenerative hyperplasia	4/16 (25.0)	2/16 (12.5)	2/16 (12.5)
Duodenum		15 cases	7 cases	8 cases
	Lymphoplasmacytic infiltrate	10/15 (66.7)	6/7 (85.7)	4/8 (50.0)
	Increased IELs	10/15 (66.7)	6/7 (85.7)	4/8 (50.0)
	Villus atrophy	0/15	0/7	0/8
	Crypt hyperplasia	0/15	0/7	0/8
	fibrosis	0/15	0/7	0/8

Table 3. Immunohistochemical, Genetic, and Serologic Findings in TPHAbs+ Patients

Patient No.	TPHAbs Index	GI Dysfunction	AIRE Gene Mutations	Gastric EC[a] (Antrum/ Body)	Duodenal EC[a]	Serotonin (0.28 –1.7 μmol/L)	Gastric CGA+ Cells[a] (Antrum/ Body)	Duodenal CGA+ Cells[a]
1	163.4	Constipation	R257X/R257X	0/0	0	<0.02	0/0	0
2	176.3	Constipation	R203X/R203X	0/0	0	0.12	130/44	45
3	116.4	Constipation	P252L/W78R	0/0	0	0.20	0/0	0
4	96.7	Diarrhea	R257X/R257X	0/0	0	0.39	18/0	3
5	238	Diarrhea	R257X/R203X	0/0	0	<0.02	nt/nt	nt
6	128.5	Diarrhea	IVS1 + 1G>C + 5delG	0/0	nt	<0.02	0/0	nt
7	94.5	Constipation	R203X/R203X	0/0	8	0.16	24/131	48
8	162.4	Diarrhea	W78R/W78R	0/0	4	0.35	nt/nt	nt
Mean ± SD	147.0 ± 45.0					0.16 ± 0.14		
9	148.5	No	R257X/R257X	0/0	9	1.2	39/62	5
10	101.3	No	R257X/R257X	0/0	0	0.31	0/58	88
11	205.2	No	R257X/R203X	0/0	6	0.03	nt/nt	nt
12	135.6	No	del GT/del 13	0/0	2	0.42	117/6	34

13	91.3	No	R257X/G227	0/0	4	1.21	5/0	2
14	129.3	No	del 13/del 13	0/0	3	0.58	0/0	5
15	83.6	No	R257X/R257X	0/24	18	0.17	0/0	10
16	74.4	No	del 13/del 13	8/6	57	0.68	108/282	73
Mean ± SD	121.2 ± 40.3					0.58 ± 0.41		
17	neg	No	R257X/C322X	22/17	77	0.38	81/65	128
18	neg	No	W78R/W78R	12/49	63	1.13	nt/nt	nt
Control group (6 cases)	neg	No	Mean ± SD	27 ± 9 16 ± 8	66 ± 19		63 ± 36 80 ± 46	94 ± 43

Abbreviations: GI, gastrointestinal; neg, negative; nt, not tested.

[a] Results are expressed as number of positive cells per 50 crypts

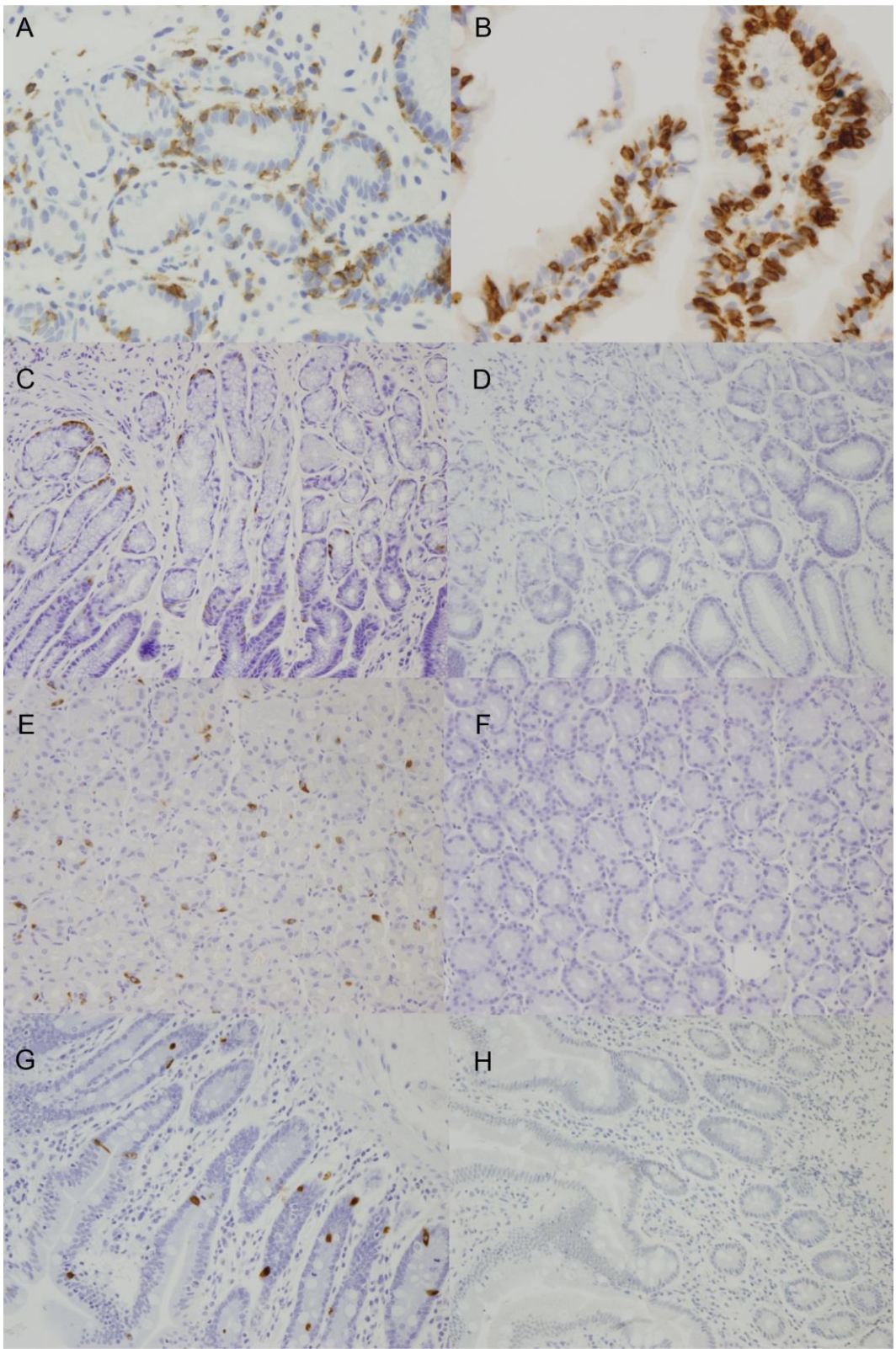

Figure 1. Immunohistochemistry of antral gastric mucosa [A: CD8 (x320)] and duodenal mucosa [B: CD8 (x320)] showing increased CD8+ T cells in the intraepithelial infiltrate. Serotonin staining of normal gastric antral (C), body (E), and duodenal (G) mucosa showing a normal distribution of EC within the glands, within the crypts, and along the villous surface (x160). Serotonin staining of gastric antrum (D), body (F), and duodenum (H) of TPHAbs+ patient with GID (x160): the gastric glands, duodenal crypt, and villus architecture are normal; however, there is a severe and almost complete loss of EC.

Figure 2. Serotonin serum levels in the 16 TPHAbs+ patients who had endoscopy (8 with GID and 8 without GID) and in 10 TPHAbs- controls without GID. X indicates the value in each patient. Broken line shows cutoff value for normal range (0.28 µmol/L) and f indicates mean value in each subgroup. In TPHAbs+ patients with GID mean serum level was significantly lower than controls and normal range, whereas in patients without GID was within the reference normal range and lower than controls.