Antiadenohypophysis Autoantibodies in Patients with Nongluten-related Gastroenteropathies

Rocío Aguado,¹ Silvia Fernández,² Orlando A. Estévez,¹ Manuel Santamaría,^{1,2†*} and Consuelo Ortega^{2†}

¹Clinical Immunology Service, University Hospital Reina Sofía, Córdoba, Spain

²Department of Immunology, Faculty of Medicine, University of Córdoba, Córdoba, Spain

Aim: To investigate the presence of antipituitary antibodies (APA) in the serum of patients undergoing gastroenteropathies (GEP) other than celiac disease (CD). **Methods:** APA were analyzed in GEP patients ($n = 103$), CD patients ($n = 94$), idiopathic growth hormone (GH) deficiency patients ($n = 21$), and 98 age- and sex-matched controls. Indirect immunofluorescence was performed in cryostat sections of baboon pituitary gland. **Results:** APA were detected in 30% of GEP patients, 38% of them showed failure to thrive. No significant differences were found when we compared thrive impairment in negative and positive APA GEP patients. Indeed, normal values of insulin-

like growth factor 1 were found in 93% of positive APA GEP patients. APA were detected in 52% of the CD patients. Ninety-one percent of them, in contrast to GEP group, showed significant growth impairment ($P < 0.05$) when compared to APA negative CD individuals. GH-deficient non-CD patients did not show APA. **Conclusions:** We have shown the presence of APA in patients with nongluten-related enteropathies. The presence of antipituitary autoantibodies in GEP patients does not seem to be associated with failure to thrive as it occurs in CD. J. Clin. Lab. Anal. 28:59–62, 2014. © 2013 Wiley Periodicals, Inc.

Key words: antipituitary antibodies; gastroenteropathies; celiac disease; growth impairment; pituitary

INTRODUCTION

Antipituitary antibodies (APA) are a group of autoantibodies directed against different cell types of the pituitary gland (1). These antibodies have been reported in patients with autoimmune diseases such as autoimmune thyroid disease (2–4), autoimmune hypopituitarism (5–7), and polyendocrine syndrome type I (8) and in patients with growth hormone deficiency (GHD, 9–11). Recently, the presence of APA in sera from children with newly diagnosed CD as well as patients with gluten-free diet has been reported (12). Their presence seems to be related to growth impairment because low levels of insulin-like growth factor 1 (IGF-1) have been described in both groups of patients (12). The involvement of APA in the pathogenesis of these diseases is not clear yet. In this regard, two types of immunofluorescence pattern have been described: a diffuse pattern, involving most pituitary cells and a granular cytoplasmatic pattern usually giving a scarce dots immunofluorescence staining. However, their clinical and biological significance, as well as why these autoantibod-

ies are not present in all CD patients, remains elusive (11, 13, 14).

With respect to nongluten-related enteropathies, little is known about the serological presence of APA. Consequently, the aim of the present work was to analyze the presence of APA autoantibodies in patients with nongluten-related inflammatory conditions.

Grant sponsor: Spanish Ministry of Education; Grant number: SAF2006-09991; Grant sponsor: Consejería de Salud, Junta de Andalucía, Spain; Grant number: 0156/05.

†These authors contributed equally to this article.

*Correspondence to: Professor Manuel Santamaría, Servicio de Inmunología, Hospital Universitario Reina Sofía, Avda. Menéndez Pidal S/N, 14004 Córdoba, Spain. E-mail: msantamaria@uco.es

Received 11 April 2013; Accepted 3 June 2013

DOI 10.1002/jcla.21644

Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. GEP Inclusion Criteria

Clinical	
Abdominal pain	+
Diarrhea	+
Vomiting	+
Inflammation in small bowel	+
Atrophia in small bowel	–
Laboratory	
Anemia	+
Antigliadin IgA/IgG antibodies	+
Antitransglutaminase IgA/IgG antibodies	–
HLA DQ2/DQ8	–
Vitamin deficiency disorders	–

MATERIALS AND METHODS

Subjects

A total of 103 patients (53 male/50 female; age range: 1–13 years) with gastroenteropathies (GEP) signs were enrolled in this study. The clinical and laboratory criteria were used for patients inclusion in our study. Clinical criteria included histological evidences of small bowel inflammation, vomiting, diarrhea, abdominal pain in the absence of villous atrophy, and intestinal tract infections; laboratory criteria included the presence of antigliadin antibodies and the absence of CD markers (Table 1). Ninety-four patients (50 male/44 female; age range: 1–13 years) diagnosed of CD were analyzed. CD diagnosis was based on the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria (15): clinical symptoms, presence of antitransglutaminase IgA antibodies, positivity of serum antiendomysium antibodies, and the histological evidence of total or partial villous atrophy. Human leukocyte antigen (HLA) class I (PCR-SSP, Invitrogen, Brown Deer, WI) and HLA class II (PCR-SSO, Innogenetics, Gent, Belgium) high-resolution typing was studied in all patients. To exclude false-negative antitissue transglutaminase 2 by IgA immunodeficiency, we detected serum normal levels of IgA in all patients. Also, 21 patients diagnosed with GHD were analyzed. A total of 98 healthy controls were used. Samples were obtained following protocols approved by the Institutional Review Board of Reina Sofia University Hospital (Spain); the Declaration of Helsinki Principles was followed, and patients and healthy control individual's parents gave written, informed consent. All patients participating in our study were untreated and had never received any specific treatment.

Methods

APA were evaluated in our four groups (GEP, CD, GHD, and controls) by an indirect immunofluorescence method on cryostat sections of a young baboon pituitary

gland supplied by BioSystem (Barcelona, Spain). In particular, fluorescein isothiocyanate conjugated goat anti-human IgG or IgA was used to detect the presence of APA. We considered positive specimens with a titer of at least 1/8. Antiendomysium autoantibodies were detected by an indirect immunofluorescence on monkey esophagus tissue sections (BioSystem). Immunoglobulin A and G antitransglutaminase (Elia Celikey IgA Well; ThermoFisher Scientific, Sweden) and Immunoglobulin A and G antigliadin antibodies (Varellisa Gliadin IgG/IgA Well; ThermoFisher Scientific) were detected as previously described (16) and considered positive if higher than 10 arbitrary units (AU)/ml. All parameters were measured in an ImmunoCap2500 (ThermoFisher Scientific). Immunoglobulin A (Siemens Healthcare, Marburg, Germany) antibodies were detected by nephelometry using BN-II (Siemens Healthcare). IGF-I values were assayed by Nuclear Medicine Service of Reina Sofia University Hospital.

Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS, Inc., Evaston, IL). Comparisons between categorical parameters (positive/negative) were performed using χ^2 test. *P*-values <0.05 were considered statistically significant.

RESULTS

Positive sera for APA displayed a granular intracytoplasmatic immunofluorescence pattern in most of pituitary cells (Fig. 1). In all cases, adenohypophysis but not neurohypophysis tissues were stained by positive sera. APA autoantibodies were not detected in our GH-deficient patients.

APA were found present in 30% of GEP patients (31/103), 52% of CD patients (49/94), and 2% of control group (2/98, Table 2). When we studied the prevalence of APA in these groups, we observed statistical significant differences when both GEP and CD patients were compared to control individuals. Also, significant differences were found while comparing GEP group and CD patients (Table 2) but not when these groups were compared regarding gender (GEP: 17M/14F; CD: 23M/26F). We also analyzed the class of APA antibodies. We detected that 81% of CD patients and 83% of GEP patients showed IgG class APA antibodies, 6% of CD patients and 16% of GEP patients showed IgA class APA antibodies, and 12% of CD patients showed IgG and IgA class APA (Table 2).

Finally, 38% of the GEP group displaying APA antibodies undergoes failure to thrive (12 patients out of 31), that was also observed in 27% of GEP patients negative for APA antibodies (19 patients out of 72). Thus, no

Fig. 1. Immunofluorescence in sera from GEP and healthy subjects were analyzed by indirect immunofluorescence. A speckled cytoplasmatic pattern is observed in GEP patients.

TABLE 2. Detection of Antipituitary Autoantibodies and Their Isotypes

	APA+	IgG	IgA	IgG + IgA
GEP	30 (%)	83 (%)	16 (%)	0 (%)
CD	52 (%)	81 (%)	6 (%)	12 (%)
Controls	2 (%)	100 (%)	0 (%)	0 (%)
GEP vs. Controls	$P < 0.05$			
CD vs. Controls	$P < 0.05$			
GEP vs. CD	$P < 0.05$			

TABLE 3. Association of Growth Impairment and Antipituitary Autoantibodies

Patients with growth impairment	APA+	APA–	<i>P</i> -value
Gastroenteropathies (GEP)	38 (%)	27 (%)	$P > 0.05$
Celiac disease (CD)	91 (%)	9 (%)	$P < 0.05$

significant differences ($P > 0.05$) were observed that could be attributed to the presence of APA autoantibodies in this group of patients, in sharp contrast with results obtained from CD patients. Indeed, failure to thrive was observed in 91% of CD patients with APA autoantibodies (Table 3). Accordingly, when we assayed IGF-I values within the APA positive GEP group, normal levels of IGF-I were observed in 93% of these patients (data not shown).

DISCUSSION

The presence of APA in healthy and disease conditions has been reported, revealing the spectrum complexity of pituitary autoimmunity (2–5, 8, 12). We have extended the analysis of APA presence to GEP and others, which were different from CD. We enrolled patients with clin-

ical signs that were different from celiac patients. These GEP patients showed inflammation of small bowel and high values of antigliadin IgA/IgG, but not positivity of antitransglutaminase IgA antibodies and non-HLA DQ2/DQ8. These clinical features allow us to classify another group of gastroenteropathies where the only presence of antigliadin could be causing inflammation but no atrophy.

In this report, we show that antipituitary autoantibodies can be detected in patients with small bowel inflammatory diseases and CD. This suggests that antiadenohypophysis antibodies do not seem to be specific markers in CD. However, our data suggested that the presence of APA in patients with GEP neither has a direct relationship with growth development nor with IGF-I levels modifications. We have detected significant differences in growth impairment in celiac patients with APA⁺ or APA[–] in accordance with recent reports (12, 17).

Thus the role of APA, or just its presence in some GEP, is controversial. The lack of expression of APA with failure to thrive in GEP other than CD allows the debate of the etiopathogenic association of APA with GEP. Significantly, autoantigen(s) recognized by APA has not been elucidated yet (18–20). In this sense, GH-producing cells have been suggested as one of the potential autoantigen(s) recognized by APA (9, 11). However, preliminary data reported here point to other autoantigens as sera from GH-deficient individuals examined did not contain APA antibodies. With respect to the relationship between GEP and APA, the data showed in this report could be indicating that a different autoantigen, with respect to CD, would be recognized in nongluten-related enteropathies by antipituitary autoantibodies. This difference of autoantigens recognized by APA could explain the different clinical signs and symptoms in both types of GEP. Thus, inflammation of certain small bowel sections rich

in endocrine cells of the Amine Precursor Uptake Decarboxylase (APUD) system could be a good candidate to be marked by APA. Therefore, elucidation of the role of APA in inflammatory small bowel diseases may benefit patients by providing new basis for an earlier therapeutic intervention.

Altogether, the facts reported here support the idea of a shared epitope between small bowel and pituitary gland as a common pattern of immunofluorescence staining was observed in all samples studied. Indeed, new tools for diagnosis and more precise profiling of GEP are already available. Consequently, these patients would benefit from more personalized therapies. In conclusion, our work shows the presence of antiadenohypophysis autoantibodies in nongluten-related GEP. We also support that these autoantibodies are not pathogenically associated with growth failure in this type of GEP, suggesting that a different feature could be responsible for different clinical manifestations between CD and nongluten-related enteropathies. The elucidation of the functional role of APA autoantibodies in human inflammatory small intestine guarantees further investigation in this field.

ABBREVIATIONS

APA	= antipituitary antibodies
CD	= celiac disease
GEP	= gastroenteropathies
GH	= growth hormone
GHD	= growth hormone deficiency
IGF-1	= insulin-like growth factor 1

REFERENCES

1. Caturegli P, Lupi I, Landek-Salgado M, Kimura H, Rose NR. Pituitary autoimmunity: 30 years later. *Autoimmun Rev* 2008;7(8):631–637.
2. Manetti L, Lupi I, Morselli LL, et al. Prevalence and functional significance of antipituitary antibodies in patients with autoimmune and non-autoimmune thyroid diseases. *J Clin Endocrinol Metab* 2007;92(6):2176–2181.
3. Kobayashi I, Inukai T, Takahashi M, et al. Anterior pituitary cell antibodies detected in Hashimoto's thyroiditis and Graves' disease. *Endocrinol Jpn* 1988;35(5):705–708.
4. Nishino M, Yabe S, Murakami M, Kanda T, Kobayashi I. Detection of antipituitary antibodies in patients with autoimmune thyroid disease. *Endocr J* 2001;48(2):185–191.
5. Nishiki M, Murakami Y, Ozawa Y, Kato Y. Serum antibodies to human pituitary membrane antigens in patients with autoimmune lymphocytic hypophysitis and infundibuloneurohypophysitis. *Clin Endocrinol (Oxf)* 2001;54(3):327–333.
6. De Bellis A, Bizzarro A, Bellastella A. Pituitary antibodies and lymphocytic hypophysitis. *Best Pract Res Clin Endocrinol Metab* 2005;19(1):67–84.
7. de Graaff LC, De Bellis A, Bellastella A, Hokken-Koelega AC. Antipituitary antibodies in dutch patients with idiopathic hypopituitarism. *Horm Res* 2009;71(1):22–27.
8. Bensing S, Fetissoff SO, Mulder J, et al. Pituitary autoantibodies in autoimmune polyendocrine syndrome type 1. *Proc Natl Acad Sci USA* 2007;104(3):949–954.
9. De Bellis A, Bizzarro A, Perrino S, et al. Characterization of antipituitary antibodies targeting pituitary hormone-secreting cells in idiopathic growth hormone deficiency and autoimmune endocrine diseases. *Clin Endocrinol (Oxf)* 2005;63(1):45–49.
10. De Bellis A, Bizzarro A, Conte M, et al. Antipituitary antibodies in adults with apparently idiopathic growth hormone deficiency and in adults with autoimmune endocrine diseases. *J Clin Endocrinol Metab* 2003;88(2):650–654.
11. De Bellis A, Salerno M, Conte M, et al. Antipituitary antibodies recognizing growth hormone (GH)-producing cells in children with idiopathic GH deficiency and in children with idiopathic short stature. *J Clin Endocrinol Metab* 2006;91(7):2484–2489.
12. Delvecchio M, De Bellis A, Francavilla R, et al. Anti-pituitary antibodies in children with newly diagnosed celiac disease: A novel finding contributing to linear-growth impairment. *Am J Gastroenterol* 2010;105(3):691–696.
13. Bellastella G, Rotondi M, Pane E, et al. Predictive role of the immunostaining pattern of immunofluorescence and the titers of antipituitary antibodies at presentation for the occurrence of autoimmune hypopituitarism in patients with autoimmune polyendocrine syndromes over a five-year follow-up. *J Clin Endocrinol Metab* 2010;95(8):3750–3757.
14. Ferrante E, Giavoli C, Elli L, et al. Evaluation of GH-IGF-I axis in adult patients with coeliac disease. *Horm Metab Res* 2010;42(1):45–49.
15. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54(1):136–160.
16. Fernandez S, Molina JJ, Romero P, et al. Characterization of gliadin-specific Th17 cells from the mucosa of celiac disease patients. *Am J Gastroenterol* 2011;106(3):528–538.
17. Iughetti L, De Bellis A, Predieri B, et al. Growth hormone impaired secretion and antipituitary antibodies in patients with coeliac disease and poor catch-up growth after a long gluten-free diet period: A causal association? *Eur J Pediatr* 2006;165(12):897–903.
18. Lupi I, Broman KW, Tzou SC, et al. Novel autoantigens in autoimmune hypophysitis. *Clin Endocrinol (Oxf)* 2008;69(2):269–278.
19. Bottazzo GF, Pouplard A, Florin-Christensen A, Doniach D. Autoantibodies to prolactin-secreting cells of human pituitary. *Lancet* 1975;2(7925):97–101.
20. De Bellis A, Pane E, Bellastella G, et al. Detection of antipituitary and antihypothalamus antibodies to investigate the role of pituitary or hypothalamic autoimmunity in patients with selective idiopathic hypopituitarism. *Clin Endocrinol (Oxf)* 2011;75(3):361–366.