Digestive and Liver Disease 42 (2010) 854–859

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Alimentary Tract

HLA-DRB1*03 and DRB1*04 are associated with atrophic gastritis in an Italian population

Edith Lahner[a], Marialuisa Spoletini[b], Raffaella Buzzetti[b], Vito D. Corleto[a], Lucy Vannella[a], Antonio Petrone[b], Bruno Annibale[a,*]

[a] Digestive and Liver Disease Unit, 2nd Medical School, Sapienza University, Rome, Italy
[b] Department of Clinical Sciences, Sapienza University, Rome, Italy

ARTICLE INFO

Article history:
Received 21 July 2009
Accepted 23 April 2010
Available online 2 June 2010

Keywords:
Atrophic gastritis
Autoimmune gastritis
Gastric autoimmunity
HLA
HLA-DRB1
Pernicious anaemia

ABSTRACT

Background: Atrophic gastritis (AG) is often considered an autoimmune disorder and is associated with other autoimmune diseases. HLA-DRB1 alleles are often associated with autoimmune diseases, however HLA-DRB1 genotyping data in AG patients are lacking. The objective of the study was to evaluate the prevalence of HLA-DRB1 in AG patients.
Methods: The occurrence of HLA-DRB1 alleles was assessed in 89 Italian AG patients (69.1% female) and 313 controls (47.3% females). Genomic DNA was extracted from peripheral venous blood, PCR-coamplified for HLA-DRB1 and typed using a reverse line-blot assay.
Results: Compared to controls, prevalence of HLA-DRB1*03 (28.1% vs. 15.9%, $p = 0.01$) and HLA-DRB1*04 (25.8% vs. 14.4%, $p = 0.01$) was greater in AG patients, conferring an OR of 2.05 and 2.07, respectively. HLA-DRB1*01 occurred more frequently in controls than in AG patients (11.5% vs. 3.4%, $p = 0.01$) conferring an OR of 0.27. AG patients carrying the HLA-DRB1*03 or *04 alleles were characterised by having more frequently autoimmune thyroid disease (70.4% vs. 42.2%, $p = 0.01$) and intestinal metaplasia (86.4% vs. 62.2%, $p = 0.01$).
Conclusions: In our population, over 50% of AG patients carry the HLA-DRB1*03 or *04 alleles associated with autoimmune diseases, suggesting that this subset of AG patients has a genetic predisposition to autoimmunity.

1. Background

Atrophic body gastritis (AG) occurs in about 2% of the general population [1,2] and up to 3-fold more frequently in patients associated with autoimmune disorders such as autoimmune thyroid disease (ATD) or type I diabetes [3,4]. From a pathological point of view, AG is characterised by disappearance of the oxyntic glands leading to the loss of production of chlorhydric acid and intrinsic factor [5]. Hypochlorhydria causes the loss of feedback on gastrin production, thus hypergastrinaemia together with low pepsinogen I levels as well as the presence of ECL hyperplasia are features of AG [6,7]. Characteristic of AG is also considered the positivity of autoantibodies to gastric parietal cells and to intrinsic factor [8,9]. AG is a chronic, often silent pathological lesion, which may manifest itself clinically as iron deficiency anaemia (IDA) [10,11] or in a later stage of the disease as pernicious anaemia (PA), defined as cobalamin malabsorption caused by the inadequate secretion of gastric intrinsic factor [12].

AG is often considered an autoimmune disorder [12], but the pathogenesis of AG is probably more complex involving interactions between genetic and environmental factors. Experimental and clinical data suggest that *Helicobacter pylori* infection may be the trigger of gastric autoimmunity, and thus AG and PA [13–15], but this issue still awaits a definite clarification. A genetic predisposition to AG and PA has been suggested by its familial clustering, the presence of autoantibodies to gastric parietal cells, and the presence of AG in 20–30% of relatives of PA patients [12,16]. Concordance with respect to PA has been observed in 12 sets of monozygotic twins, strongly suggesting a genetic predisposition to the development of the disease [17]. The association of specific alleles of class II genes of the human leucocyte antigen (HLA) region, in particular at the DRB1 locus, is well established in many autoimmune diseases, such as systemic lupus erythematosus, type 1 diabetes, rheumatoid arthritis as well as ATD [18,19]. Few previous studies, performed in the early 80 s, have focused on the evidence of a link between PA and particular HLA molecules, sug-

* Corresponding author at: Dipartimento di Malattie Digestive e del Fegato, Ospedale Sant'Andrea, Universita' Sapienza Roma Ospedale Sant'Andrea, Via di Grottarossa 1035, 00189 Rome, Italy. Tel.: +39 06 80345289; fax: +39 06 4455292.
E-mail address: bruno.annibale@uniroma1.it (B. Annibale).

1590-8658/$36.00 © 2010 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.dld.2010.04.011

gesting genetic heterogeneity. Associations of PA with HLA-DR2, -DR4 and -DR5 antigens [20–23] have been reported, and in PA patients with a concomitant endocrine disease the occurrence of HLA-DR3/DR4 antigens has been observed [20]. In these studies, the HLA antigens were detected using serological assays and the study population was limited to AG patients with concomitant PA. Moreover, these studies were performed before routine gastroscopy and a histological consensus classification of gastritis was introduced [24].

To our knowledge, recent studies focusing on HLA-DRB1 genotyping in patients with biopsy-proven AG are lacking. Based on this background, the aim of the current study was to evaluate the role of HLA-DRB1 genotypes in a well-defined population of AG patients.

2. Materials and methods

2.1. Study population and design

Following a case–control study design, we compared the occurrence of HLA-DRB1 alleles between a series of AG patients from central Italy (Latium region) and an unrelated control group without AG, recruited from the same geographical region. All patients were of Caucasian origin. In addition, we investigated in a cross-sectional manner whether the AG patients carrying the most frequently observed HLA-DRB1 phenotypes were characterised by specific clinical features.

2.1.1. Patients

Overall, 101 patients (70.3% female, median age 53 [range 20–82] years) previously diagnosed with AG in our Gastroenterology Unit (an academic, tertiary-care medical institution) were included [4,10]. All AG patients had a detailed initial assessment including fasting gastrin and gastroscopy with biopsies from gastric antrum and body mucosa. They also had basal and peak acid output to pentagastrin [10]. A serum sample for the detection of antibodies against intrinsic factor, parietal cells and *H. pylori* was taken. Initial assessment included also a clinical interview, during which personal and clinical data as well as information about personal and family history, in particular regarding autoimmunity, were recorded. It was accurately verified, that all the included AG patients were certainly unrelated. Peripheral venous blood specimens for the extraction of genomic DNA and genotyping were taken from each patient. The procedure of DNA extraction gave unsatisfactory results in 12 specimens. Thus, DNA samples for HLA-DRB1 genotyping were available for 89 (88.1%) AG patients (69.7% female, median age 53.5 [range 20–82] years).

2.1.1.1. Diagnosis of AG. The presence of AG was defined on the basis of the concomitant presence of fasting hypergastrinaemia, pentagastrin-resistant hypochlorhydria, histological confirmation of body atrophy and ECL hyperplasia [25–27].

2.1.1.2. Diagnosis of PA. PA was defined as a haemoglobin concentration < 14 g/dL for men (normal range 14–18 g/dL) and 12 g/dL for women (normal range 12–16 g/dL), accompanied by MCV ≥ 100 fL, low serum cobalamin levels (normal values 190–950 pg/ml), response to vitamin B_{12} therapy, and histological confirmation of gastric body mucosa atrophy [10,27].

2.1.1.3. Diagnosis of IDA. IDA was defined as haemoglobin concentration < 14 g/dL for men (normal range 14–18 g/dL) and 12 g/dL for women (normal range 12–16 g/dL), MCV < 80 fL (normal range 80–100 fL) and low serum ferritin levels (normal range 30–180 ng/mL) [10,28].

2.1.1.4. Endoscopical, histological and serological procedures. All patients underwent gastroscopy with standardised biopsy sampling from the antrum ($n = 3$) and body ($n = 3$) mucosa for conventional histopathological examination [10]. The degree of gastritis was assessed according to the updated Sydney System [24]. Atrophy of the gastric body mucosa was defined as focal or complete oxyntic gland loss and/or their replacement by metaplastic pyloric or intestinal glands. Atrophy of the gastric antral mucosa was defined as focal or complete disappearance of antral glands and/or their replacement by intestinal metaplastic epithelium [10]. The ECL cell proliferative pattern was evaluated in non-intestinalised areas of gastric body mucosa and was assessed as described elsewhere [29]. All patients underwent serological studies. Fasting gastrin levels were evaluated by a specific radioimmunoassay (RIA) and pepsinogen I levels were measured using a commercial RIA kit (Pepsik, Sorin, Saluggia, Italy) [10]. *H. pylori* immunoglobulin G antibodies were determined using a commercial ELISA kit (GAP test IgG, BioRad, Milan, Italy). AG patients were defined as having active *H. pylori* infection when both histology and ELISA serology were positive [25].

2.1.1.5. Diagnosis of ATD. The presence of ATD was defined as previously described [4]. Briefly, the thyroid status of AG patients was evaluated on the basis of clinical and ultrasonographical examination, serum iodothyronines and basal TSH measurements, serum antiperoxidase antibodies detection, and when needed of anti-TSH receptor antibodies as well as radioiodine uptake and thyroid scan. Thyroid hormones and thyroid autoantibodies in serum were determined by commercial kits: free triiodothyronine and free thyroxine levels by radioimmunoassay (Ares-Serono, Milan, Italy); basal thyrotropin levels by immunoradiometric assay (Radim Techland, Liege, Belgium); antiperoxidase antibodies were measured by a radioligand assay (Radim Techland, Liege, Belgium) and anti-TSH receptor antibodies by a radioreceptor assay (Ares-Serono, Milan, Italy). Thyroid gland size, echogenicity of the parenchyma, and nodular lesions were evaluated by ultrasonographic examination. The diagnosis of autoimmune thyroiditis was based on the presence of antiperoxidase antibodies (antibody titres stably > 200 U/ml in at least two separate measurements) and characteristic ultrasound features (i.e., non-homogeneous pattern with diffuse reduction of echogenicity) in presence, but even in absence of mild or overt hypothyroidism [4]. Thyroid atrophy was diagnosed in the presence of reduced thyroid volume and ultrasound-detected diffuse fibrosis of the gland. Graves' disease was diagnosed in patients with clinical and biochemical hyperthyroidism presenting with anti-TSH receptor antibodies.

2.1.2. Controls

A group of control subjects, recruited from the same geographical region as the patients with AG (central Italy, Latium region) with no history of autoimmune disease, normal haemoglobin and MCV values (absence of anaemia and/or macrocytosis) and negative *H. pylori* serology (IgG ELISA, GAP test IgG, BioRad, Milan, Italy) were collected at Blood Transfusion Service of Sapienza University, Rome. All controls were of Caucasian origin. DNA samples were obtained from 313 controls (148 [47.3%] females, median age 34 [range 20–59] years).

The study was performed in a blind fashion, so that the case–control status of samples as well as patient characteristics was unknown to the investigators performing the molecular analyses.

The study was approved by the local Ethical Committees, and patients and controls provided signed informed consent.

2.2. HLA genotyping

Genomic DNA was extracted from peripheral venous blood specimens by a conventional salting-out procedure. The extracted