Significance of HLA-B*51 allele expression in Crohn's disease: a case-control study

A. Kostianovsky[1], P.E. Llanquimán[2], P.A. Olivera[3], S.A. Muñoz[4], I. Gandino[5], A.O. Orden[6], F. Dal Pra[7], S. Teper[8], M.C. Khoury[9], A.M. Sambuelli[2], A. Allievi[4]

[1]Internal Medicine Section, Department of Medicine, CEMIC University Hospital, Buenos Aires; [2]Inflammatory Bowel Disease Group, Hospital Dr. Carlos B. Udaondo, Buenos Aires; [3]Gastroenterology Section, Department of Medicine, CEMIC University Hospital, Buenos Aires; [4]Consultorio de Enfermedades Autoinmunes, Buenos Aires; [5]Hospital General de Agudos Dr. Juan A. Fernández, Buenos Aires; [6]Clínica San Camilo, Buenos Aires; [7]Instituto de Rehabilitación Psicofísica (IREP), Buenos Aires; [8]Histocompatibility Laboratory, CEMIC University Hospital, Buenos Aires; [9]Hospital Dr. Carlos B. Udaondo, Buenos Aires, Argentina.

Abstract

Objective

*Crohn's disease (CD) and Behçet's disease (BD) are two autoinflammatory diseases that share clinical and pathogenic features. Furthermore, when BD involves the gastrointestinal tract, it is extremely difficult to distinguish endoscopic lesions from CD lesions. HLA-B*51 allele expression is highly associated with BD diagnosis. In this study we analysed HLA-B*51 status in 70 Argentine patients with confirmed CD diagnosis and compared it to our previous Argentine BD cohort, with the aim of finding similarities or differences between these two diseases regarding HLA-B*51 status.*

Methods

*This is a multi-centre case-control study, including 70 patients with confirmed CD diagnosis, who underwent HLA-B*51 allele status testing; the results were compared to our previous BD cohort of 34 patients.*

Results

*Among patients with CD, 12.85% were positive for the HLA-B*51 allele, compared with 38.24% patients with BD (OR=0.238; 95% CI=0.089-0.637; p=0.004).*

Conclusion

*Our finding suggests that determination of HLA-B*51 allele status may contribute to the differential diagnosis between CD and BD.*

Key words

HLA-B*51, Crohn's disease, Behçet's disease

Alex Kostianovsky, MD
Pablo E. Llanquimán, MD
Pablo A. Olivera, MD
Sebastián A. Muñoz, MD
Ignacio Gandino, MD
Alberto O. Orden, MD
Fernando Dal Pra, MD
Sara Teper, BCHM
Marina C. Khoury, MD
Alicia Maria Sambuelli, MD
Alberto Allievi, MD

Please address correspondence to:
Alberto Allievi
Consultorio de Enfermedades Autoinmunes,
Blanco Encalada 5230 3D,
C1431CDR,
Ciudad Autónoma de Buenos Aires, Argentina.
E-mail: beto.allievi@gmail.com

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Introduction

Ulcerative colitis and Crohn's disease (CD) are the major inflammatory bowel diseases. Both are characterised by chronic inflammation of the gastrointestinal tract (1). Although CD usually affects several locations of the gastrointestinal tract, small bowel CD may also occur; in such cases, tuberculosis, lymphoma, and Behçet's disease (BD) are differential diagnoses to be ruled out (2-4).

BD is a chronic, recurrent, inflammatory disorder typically characterised by oral and genital ulcers and skin lesions; however, other serious manifestations may also occur, including ocular, large vessel, neurological and gastrointestinal involvement (3, 5). BD may affect any site of the gastrointestinal tract (ulcers appearing mainly in the terminal ileum and cecum) (6), with the most common symptoms being bleeding, diarrhoea, nausea and vomiting, abdominal pain and weight loss (7). The pathological findings of the disease include vascular injury, hypercoagulability, hyperfunction of neutrophils, and autoimmune response (2, 8). HLA-B*51 allele expression is strongly associated with BD diagnosis (9). We have validated this finding in an Argentine BD cohort (10). Interestingly, CD and BD share several clinical features, such as ocular (*i.e.* uveitis) and mucocutaneous (*i.e.* erythema nodosum and oral ulcers) involvement. Furthermore, in case of BD involving the bowel, it may be extremely difficult to distinguish lesions (both endoscopically and pathologically) from CD lesions (11). In terms of genetic HLA background, CD is associated with HLA-DRB1*04 (12).

In view of these clinical similarities, our aim was to investigate the prevalence of HLA-B*51 allele expression in a CD cohort, and to compare it to our BD cohort previously studied (10).

Materials and methods

Study design and participants

We conducted a multi-centre case-control study. Seventy consecutive patients with confirmed (pathologically proven) CD diagnosis were included from 3 tertiary hospitals (Hospital Dr. Carlos B. Udaondo, CEMIC University Hospital and Hospital General de Agudos Dr. Juan A. Fernández) in Buenos Aires, Argentina, between January 2020 and April 2021. Their results based on demographic and clinical features, as well as HLA-B*51 status, were compared to our previous BD cohort of 34 patients (10). Patients' demographic and clinical features were recorded in a database by referring physicians.

HLA typing

For HLA class I cases, typing was performed with a polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO) coupled with a Luminex platform using a medium resolution LABType SSO class I A and B locus typing test (One Lambda Inc., California, USA) according to the manufacturer's instructions.

Statistical analysis

In view of our previous sample size of 34 BD patients, we recruited 70 CD patients (CD:BD ratio 2:1). Power of 76.22% was calculated with the OpenEpi® online program for a 2-tail alpha of 0.05 assuming a probability of detection HLA-B*51 of 14.5% in CD (similar to healthy controls) and 38.2% in BD, as previously shown (10). Descriptive statistics including percentages, mean, standard deviation (SD), median and range were calculated. Characteristics between CD and BD groups, and between HLA-B*51 positive and negative patients were compared using Fisher's exact t-test.

The frequencies of the alleles and genotypes among patients and controls were compared by chi-square tests. Odds ratios and 95% confidence intervals were calculated. A p-value of <0.05 was considered significant in all analyses. Statistical analysis was made using Stata 14.0® (StataCorp 2015, TX, USA).

The study was approved by local ethical committees of all three hospitals. Study participants gave their written informed consent to participate.

Results

Seventy CD patients were included in the analysis. Demographic and clinical variables are shown in Table I. All 70 patients had a diagnosis of CD confirmed

Table I. Demographic and clinical features of CD patients.

Mean age ± SD (years)	41.5 ± 15.5
Gender	
Male (n, %)	44 (62.85%)
Female (n, %)	26 (37.15%)
Pathologically confirmed CD (n, %)	70 (100%)
Small bowel involvement	
Yes (n, %)	30 (42.85%)
No (n, %)	40 (57.15%)
Large bowel involvement	
Yes (n, %)	60 (85.7%)
No (n, %)	10 (14.3%)
Biologic agent treatment	
Yes (n, %)	51 (72.86%)
No (n, %)	19 (27.14%)

Table II. Distribution according to Montreal classification.

	n
Age at diagnosis (years)	
A1 (<16)	12
A2 (17-39)	45
A3 (>40)	13
Location	
L1 (ileal)	9
L1+L4 (L1 + upper GI tract)	1
L2 (colonic)	40
L3 (ileocolonic)	19
L3+L4 (L3 + upper GI tract)	1
Behaviour	
B1 (non-stricturing, non-penetrating)	34
B1p (B1 + concomitant perianal disease)	18
B2 (stricturing)	6
B2p (B2 + concomitant perianal disease)	5
B3 (penetrating)	5
B3p (B3 + concomitant perianal disease)	2

by biopsy (62 endoscopic and 8 surgical samples). Mean age was 41.5 years and male gender accounted for 62.6% of patients. Most of the patients were treated with biologic agents at some point during their disease course.

Montreal's classification distribution is shown in Table II, with most patients having colonic involvement, 7 patients fistulising disease and 11 stricturing behaviour.

Nine out of 70 (12.85%) patients with CD were positive for the HLA-B*51 allele, compared with 13 of 34 (38.24%) patients with BD (OR= 0.238; 95%CI= 0.089-0.637; p=0.004). Regarding the BD cohort, all 34 patients had a definitive diagnosis of BD according to the International Study Group for Behçet Disease Criteria (20 patients had 3 criteria, 13 patients had 4 criteria and 1 patient had 5 criteria) and none of them had gastrointestinal involvement of the disease (10).

Table III. Clinical manifestation in CD patients according to HLA-B*51 status.

Manifestation, n (%)	HLA-B*51 (+) (n=9)	HLA-B*51 (-) (n=61)	p
Oral ulcers	2 (22.2)	13 (21.3)	0.621
Skin involvement	2 (22.2)	20 (32)	0.415
erythema nodosum	0	12 (19.67)	0.164
pseudo folliculitis	1 (11)	3 (4.92)	0.431
acneiform lesions	0	6 (9.84)	0.423
Genital ulcers	1 (11)	2 (3.28)	0.343
Anterior uveitis	0	4 (6.56)	0.569
Conjunctivitis	1 (11)	5 (8.20)	0.577

Table IV. Clinical manifestations: comparison between CD and BD patients.

	CD (n=70)	BD (n=34)	p
Oral ulcers	15 (21.43%)	34 (100%)	<0.001
Skin involvement	22 (31.43%)	30 (88.24%)	<0.001
erythema nodosum	12 (17.14%)	12 (35.29%)	0.037
pseudo folliculitis	4 (5.71%)	15 (44.12%)	<0.001
acneiform lesions	6 (8.57%)	14 (41.18%)	<0.001
Genital ulcers	3 (4.29%)	23 (67.65%)	<0.001
Anterior uveitis	4 (5.71%)	10 (29.41%)	0.002
Posterior uveitis	0	10 (38.24%)	<0.001
Conjunctivitis	6 (8.57%)	7 (20.59%)	0.080

No significant differences were observed in clinical manifestations within the CD group according to HLA-B*51 status (Table III), including site of gastrointestinal involvement (small bowel disease 3/30 and colonic disease 6/40).

When comparing extraintestinal clinical manifestations shared by CD and BD, we observed a higher frequency of all of them (except for conjunctivitis) in BD (Table IV). Out of the 34 BD patients, 3 (8.8%) had CNS involvement and 24 (70.5%) joint involvement (data not shown).

Discussion

The association between BD and HLA-B*51 has been widely demonstrated worldwide (9). We have validated this association in Argentine patients with BD. Of 34 patients with BD, 38.2% were positive for HLA-B*51 compared to 14.5% of 240 healthy controls (OR=3.75; 95%CI=1.71-8.19; p=0.0012) (10).

Meta-analysis and genome wide association studies showed no association of HLA-B*51 as a susceptibility locus for CD. However, a specific locus called ERAP-1 (endoplasmic reticulum aminopeptidase 1) was found to influence peptides trimming for presentation in a class 1 MHC context and regulating HLA-B*51 activity(13)(14). In addition, IL-10 gene polymorphisms also account for genetic susceptibility in CD and BD (15).

Considering these genetic data and given that CD and BD may share similar clinical manifestations, we decided to evaluate the frequency of HLA-B*51 allele expression in a group of CD patients, and to compare it with our previous BD data. We found that HLA-B*51 allele expression was significantly lower in CD patients than in BD patients (12.85% *vs*. 38.24% respectively), and similar to its expression in healthy controls (14.16%), as we have previously shown (10). In summary, our data suggests that similarities between CD and BD are not associated with individual HLA-B*51 expression.

We found no significant differences in clinical features according to HLA-B*51 presence or absence in CD patients, probably due to their low incidence of HLA-B*51 expression. Interestingly, the incidence of extraintestinal manifestations of CD shared with BD was low in our cohort, which could probably define a phenotype of local

CD. However, this finding may present a selection bias since most of the patients herein included were followed by the Division of Gastroenterology in our hospitals. Regarding mucocutaneous involvement, oral and genital ulcers where obviously more prevalent in BD than in CD. As expected, oral ulcers were more prevalent than genital ulcers in CD patients, the latter being an uncommon but know extraintestinal CD feature also termed as "metastatic CD", more frequently present in women (16). Genital ulcers occurred in 3 female patients in our CD cohort: 1 in the group of 9 HLA-B*51 positive patients (11%) and 2 in the group of 61 HLA-B*51 negative patients (3.2%). Although there is no statistical significance in this finding, and it could therefore be a tendency by chance, it could also be expression of the common underlying physiopathologic processes shared by CD and BD, where HLA-B*51 expression is supposed to play a role (14).

This study has some limitations. Case control studies may be biased to underdetect subpopulations in genetic association studies. Our sample size is small and was calculated to draw a comparison with our previous BD study, and it includes CD patients with confirmed diagnosis. Since this is a local study, our data should be extrapolated cautiously. However, as it is a multi-centre study including private and public hospitals (one being a national referral centre for GI diseases), our sample seems to be representative of CD in the Argentine population.

In conclusion, our findings suggest that the determination of HLA-B*51 allele status may contribute to the differential diagnosis between CD and BD with gastrointestinal involvement, since HLA-B*51 is not over expressed in our CD cohort, as opposite to BD patients. Future studies at a larger scale are needed to confirm the usefulness of HLA-B*51 status for differential diagnosis between CD and BD with gastrointestinal involvement in those cases where clinical features and endoscopic finding make them indistinguishable. Meanwhile, we consider that our findings add interesting data to an uncertain clinical scenario.

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