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Study of killer immunoglobulin-like receptor genes and human leukocyte antigens class I ligands in a Caucasian Brazilian population with Crohn's disease and ulcerative colitis

Timothy J. Wilson [a], Mariana Jobim [a,b,*], Luiz Fernando Jobim [b,c], Pamela Portela [a], Patrícia H. Salim [a], Mário A. Rosito [d], Daniel C. Damin [d], Cristina Flores [e], Alessandra Peres [f], Marta Brenner Machado [g], José Artur Bogo Chies [h], Gilberto Schwartsmann [c,i,j], Rafael Roesler [i,j,k]

[a] *Postgraduate Course in Medical Sciences, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[b] *Department of Immunology, Hospital de Clínicas de Porto Alegre, Brazil*
[c] *Department of Internal Medicine, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[d] *Department of Coloproctology, Hospital de Clínicas de Porto Alegre, Brazil*
[e] *Department of Gastroenterology, Hospital de Clínicas de Porto Alegre, Brazil*
[f] *Centro Universitário Metodista IPA, Porto Alegre, Brazil*
[g] *Department of Gastroenterology, Hospital São Lucas da PUCRS, Brazil*
[h] *Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[i] *Cancer Research Laboratory, University Hospital Research, Center (CP-HCPA), Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[j] *National Institute for Translational Medicine (INCT-TM), Porto Alegre, RS, Brazil*
[k] *Laboratory of Molecular Neuropharmacology, Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*

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ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory diseases of the bowel, of unknown origin. Exposure to specific environmental factors by genetically susceptible individuals, leading to an inadequate response of the immune system, is one of the potential explanations for the occurrence of these diseases. Natural killer cells are part of the innate immune system recognizing class I HLA (human leukocyte antigen) molecules on target cells through their membrane receptors. The main receptors of the natural killer cells are the killer immunoglobulinlike receptors (KIRs). Our study aimed to evaluate the association between the KIR genes in patients with inflammatory bowel diseases and healthy controls. We typed 15 KIR genes and HLA class I ligands in 248 unrelated Brazilian Caucasians, of which 111 had UC and 137 had CD, and 250 healthy controls by polymerase chain reaction using sequence-specific oligonucleotides and sequence-specific primers. We found an increase in KIR2DL2 in controls (inflammatory bowel disease [IBD]: $p < 0.001$; UC: $p = 0.01$; CD: $p =$ not significant [NS]). The genotype 2DL2+/HLA-C lys(80)+ was also more common in controls (IBD: $p = 0.005$; UC: $p = 0.01$; CD: $p =$ NS); as well as 2DL1+/HLA-C Asn(80)+ (IBD: $p = 0.026$; UC: $p =$ NS;CD: $p =$ NS). The imbalance between activating and inhibitory KIR and HLA ligands may explain, at least in part, the pathogenesis of these inflammatory bowel diseases.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main forms of chronic inflammatory bowel diseases (IBD), and although having an unknown etiology, are believed to result from a complex interaction of several genes conferring risk of disease and environmental factors, leading to an inadequate inflammation response [1].

Recent evidence suggests that innate immune response plays an important role in initiating the inflammatory cascade and subsequent characteristic pathologic adaptive immune responses in IBD. The normal mucosal barrier on the bowel wall separates the host from immunologic challenges represented by bacteria, viruses, fungi, and food antigens within the lumen. Innate immune mechanisms are involved in this relationship, allowing a coexistence with commensal agents while contributing to protect the host from invasion by a rapid response to pathogens. In IBD patients there is a break in this tolerance and inflammation supervenes driven by the intestinal microbial flora [2,3].

Activation of innate immunologic response relies on the recognition by the host of a series of microbial compounds through a variety of receptors called pattern recognition receptors, which include the toll-like receptor (TLR) and the NOD (nucleotide-binding oligomerization domain) receptor families (a family of intracellular

* Corresponding author.
E-mail address: mjobim@hcpa.ufrgs.br (M. Jobim).

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bacterial sensors) [4–7]. Mutations in these receptors have been associated with the development of IBD [8–16].

Several studies have implied the susceptibility of human leukocyte antigens (HLA) in determining inflammatory bowel disease. A meta-analysis of all genetic linkage studies was published in 2004, confirming the relationship between IBD and HLA molecules [17]. Previous studies have shown a link of HLA class II to UC and CD, revealing a possible connection between DR13 with UC and DR3 with DC [18]. A recent publication further suggests a possible increased risk of developing colorectal cancer in those who have UC and carry DR17 [19].

The natural killer (NK) cells are also key components of the innate immune system and participate in the early response against infected or transformed cells, through the production of cytokines and by direct toxic effect [20,21]. Their function is regulated by a variety of activating and inhibitory receptors, which are expressed on the cell surface. HLA class I molecules are located on the surface of target cells and are recognized by NK cell immunoglobulinlike receptors (KIRs) [20].

The KIR receptor gene family is located on a region called IBD6, on chromosome 19q13.4, and contains 15 genes and two pseudogenes. KIR molecules interact with the HLA-C and HLA-B alleles, providing information on the HLA class I surface expression of target cells. Engagement of KIR with the appropriate HLA ligand induces either inhibitory or activating signaling depending on the presence of intracellular immunoregulatory tyrosine– based inhibitory or activating motifs. Both KIR and their ligands display considerable genetic diversity. There is a growing interest about the possible role of KIR genes in several diseases, particularly those with alteration of the inflammatory response. A hypothesis is that various KIR/HLA combinations could produce differences in NK/NK T-cell activation [22,23].

Dimorphisms in the HLA-Cw α1 domain, characterized by amino acid positions Ser77/Asn80 and Asn77/Lys80, define serologically distinct allotypes of HLA-Cw (Cw group 1 and group 2, respectively). Group 1, or C1, consists of HLA-Cw1, HLA-Cw3, HLA-Cw7, HLA-Cw8, HLA-Cw13, and HLA-Cw14; and group 2, or C2, consists of HLA-Cw2, HLA-Cw4, HLA-Cw5, HLA-Cw6, HLA-Cw17, and HLA-Cw 18. KIR2DS2, KIR2DL2 e-KIR2DL3 recognize C1, whereas KIR2DS1 and KIR2DL1 recognize C2. KIR3DL1 is identified by HLA-Bw4. Although there is still some controversy on the recognition of KIR3DS1, the current consensus is that KIR3DS1 is not recognized by Bw4 or Bw4I80 alleles. There is some indication of joint activity between KIR3DS1 with select Bw4s, such as B*57 and B*58, leading to enhanced NK cell activity, but it remains unknown if B*57 or B*58 can directly recognize KIR3DS1 [24].

Up to now, 15 KIR genes and pseudogenes were identified. Most studies on KIR genes have focused on populations from Europe, Asia, and North America, whereas Central and South America, Oceania, and Africa remain poorly studied [25,26]. Because of KIR specificity for HLA class I allotypes, and their extensive polymorphisms, it is reasonable to imagine that KIR gene variation affects resistance and susceptibility to several diseases with an autoimmune basis, such as psoriasis, psoriatic arthritis, rheumatoid arthritis, diabetes among others [21,22]. Genetic studies of the association of KIR with disease have been concerned with viral infections, solid tumors, hematological disease, and inflammatory diseases such as IBD [21]. The modulation of NK activity may establish a possible regulatory mechanism in gut inflammation. In a recent publication, Jones *et al.* found an increase in 2DL2 and 2DS2 in patients with UC, suggesting that KIR genotype and HLA ligand interaction may contribute to the genetic susceptibility to this disease [27].

In the present study, we examined 15 KIR genes and HLA ligands in a group of IBD patients and compared with healthy controls, aiming at the identification of patterns of KIR genotypes and HLA ligands that could be more associated with susceptibility to these diseases. To the best of our knowledge, this is the first study of KIR genes in a Brazilian Caucasian population with CD and UC.

2. Subjects and methods

2.1. Patient and control samples

A total of 248 patients from Hospital de Clínicas de Porto Alegre and Hospital São Lucas, from PUCRS (110 men and 138 women) with IBD and 250 ethnically matched healthy controls (125 men and 125 women) from the Brazilian bone marrow center were studied for KIR polymorphism. The age was 40.19 $\pm$ 12.01 (mean $\pm$ SD) years for the patients and 38.58 $\pm$ 10.49 years for the controls.

Blood samples were collected after obtaining an informed consent and authorization of the ethical committee in accordance with the Declaration of Helsinki [28]. The diagnosis of IBD was based on standard clinical, radiological, and histologic criteria [29], and in accordance with the recent consensus on IBD published by the European Crohn's and Colitis Organization [30,31]. Patients with other types of colitis were excluded from the study. Both groups were tested for KIR and ligand gene frequencies.

2.2. DNA extraction and polymerase chain reaction

Blood samples were collected into tubes containing ethylenediamine tetraacetic acid. DNA was extracted using salting-out procedure [32]. DNA samples were genotyped using polymerase chain reaction (PCR)-sequence-specific primers for 15 KIR genes (2DS1, 2DS2, 2DS3, 2DS5, 3DS1, 2DS4, 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2 e 3DL3, 2DP1). The PCR primers and conditions were based on previous reports [33]. Internal control was included in each PCR reaction. The combination employed to achieve a 10 μl volume reaction was 10 ng of genomic DNA, 500 nmol/l specific primers, 2.5 U of Taq polymerase, 0.08 μl of PCR buffer, 0.3 μl MgCl, and 10 μl of distilled water, which was amplified by the GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, CT).

Temperature and cycling conditions for PCR reaction were as follows: denaturation for 3 minutes at 94°C, followed by four cycles of 15 seconds at 94°C, 15 seconds at 65°C, 15 seconds at 72°C; 21 cycles of 15 seconds at 94°C, 15 seconds at 60°C, 30 seconds at 72°C; five cycles of 15 seconds at 94°C, 1 minute at 55°C, 2 minutes at 72°C, and a final elongation step at 72°C for 7 minutes. Resulting products were visualized under ultraviolet light after electrophoresis in 1% agarose gels containing ethidium bromide. HLA typing A, B, and Cw alleles was also done using PCR with specific primers, as described before [34].

2.3. Statistical analysis

Pearson χ^2 test with continuity correction was used for comparison of the KIR gene frequency with the control group, and in some, where the expected difference between the two groups was small, Fisher's exact test was used. Odds ratio, confidence interval, and significance values were calculated using SPSS 16.0. Bonferroni correction was used to adjust for the number of genes used.

3. Results

From the IBD clinic 248 patients were recruited137 with CD and 111 with UC. The frequency of the inhibitory gene KIR2DL2 was increased in controls in comparison with IBD patients and UC group (IBD: $p < 0.001$; UC: $p = 0.01$; CD: $p =$ not significant [NS]). No difference was seen between the controls and the cases with regard to HLA ligand A3, A11, Bw4, C1, and C2 (Table 1).

We further analyzed the combination of inhibitory and activating KIR genes with or without their corresponding HLA-C ligands. The reason for making this association was to evaluate the effect of genetic variation at the KIR locus in combination with genes encoding their HLA-C ligands on disease susceptibility or protection. The