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Reduced frequency of two activating KIR genes in patients with sepsis

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ABSTRACT

Natural killer (NK) cell activity is regulated by activating and inhibitory signals transduced by killer cell immunoglobulin-like receptors (KIR). Diversity in KIR gene repertoire among individuals may affect disease outcome. Sepsis development and severity may be influenced by genetic factors affecting the immune response. Here, we examined sixteen KIR genes and their human leucocyte antigen (HLA) class I ligands in critical patients, aiming to identify patterns that could be associated with sepsis. Male and female patients (ages ranging between 14 and 94 years-old) were included. DNA samples from 211 patients with sepsis and 60 controls (critical care patients with no sepsis) collected between 2004 and 2010 were included and genotyped for KIR genes using the polymerase chain reaction method with sequence-specific oligonucleotide (PCR-SSO), and for HLA genes using the polymerase chain reaction method with sequence-specific primers (PCR-SSP). The frequencies of activating KIR2DS1 and KIR3DS1 in sepsis patients when compared to controls were 41.23% *versus* 55.00% and 36.49% *versus* 51.67% ($p = 0.077$ and 0.037 respectively before Bonferroni correction). These results indicate that activating KIR genes 2DS1 and 3DS1 may more prevalent in critical patients without sepsis than in patients with sepsis, suggesting a potential protective role of activating KIR genes in sepsis.

1. Introduction

Sepsis can be defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. During sepsis, early activation of immune cells occurs, and the imbalance between pro-inflammatory and anti-inflammatory pathways culminate in organ dysfunction [1,2]. The precise mechanisms by which sepsis produces an uncontrolled inflammatory response and multiple organ dysfunction remain unknown, but several studies have suggested the involvement of genetic factors [3,4].

Natural killer (NK) cells are key lymphocytes involved in early immune response against infected or transformed cells in clinical conditions including infections, tumors, and allogeneic hematopoietic cell transplantation, while remaining tolerant to self [5,6]. NK cells can play a role in sepsis stimulating both pro- and anti-inflammatory responses, for example by activating phagocytic cells at the site of infection and producing and releasing interferon-γ (INF-γ), leading to macrophage activation. NK cells can also be activated by antigen-presentation cells, such as dendritic cells, thus amplifying the inflammatory response [7,8].

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The activity of NK cells is regulated by the balance between signals transduced from activating and inhibitory cell surface receptors, including killer immunoglobulin-like receptors (KIR), which are members of the immunoglobulin superfamily [9,10]. Inhibitory KIR molecules bind to target HLA class I molecules and prevent the attack of NK cells on normal cells. When an activating KIR binds to its ligand, activating signals are generated, leading to the destruction of target cell [11–13]. Genes encoding KIR are located on human chromosome 19q13.4 in the leucocyte receptor complex. To date, seventeen KIR receptors have been characterized in humans: eight inhibitory types (2DL1-3; 2DL5A and B; 3DL1-3), seven activating types (2DS1-5, 3DS1, 2DL4), and two pseudogenes that do not encode a functional KIR receptor (2DP1 and 3DP1). Of these, four are always present and are considered framework genes (2DL4, 3DL2, 3DL3, 3DP1) [13–16]. The ligands for KIR are the classical HLA class I molecules HLA-A, -B and -C. Based on the dimorphism in the epitope at position 80, all HLA-C alleles can be divided into two groups: C1 group carrying asparagine, and the C2 group carrying lysine at this position. The receptors KIR2DL2, KIR2DL3 and KIR2DS2 bind HLA-C1 ligands, whereas KIR2DL1 and KIR2DS1 bind HLA-C2 ligands. The inhibitory KIR3DL1 recognizes HLA-B Bw4 allotypes and KIR3DL2 binds HLA-A3 and HLA-A11 [17].

Displaying specificity for HLA class I allotypes, and extensive polymorphism variation, KIR gene variation affects resistance and susceptibility to a great number of diseases in which the involvement of the immune system is determinant. Genetic susceptibility or resistance to infectious and inflammatory disorders, together with environmental and host risk factors, is thought to determine disease progression [18,19]. There are a number of studies describing a relationship between KIR genes and several diseases but no previous studies have has examined the possible role of KIR in sepsis. In the present study, we examined sixteen KIR genes and their HLA class I ligands in a group of 271 critical patients aiming at the identification of patterns in KIR genotypes and HLA ligands that could be associated with sepsis.

2. Methods

2.1. Patient and control samples

DNA samples of two hundred and seventy-one critical care patients of both genders, with ages ranging from 14 to 94 years old, admitted to the Intensive Care Unit, São Lucas Hospital, Pontifical Catholic University of Rio Grande do Sul, in Porto Alegre, Brazil, in the period between 2004 and 2010 were included. Their blood was collected into tubes containing EDTA (ethylenediamine tetraacetic acid) and DNA extracted using a salting-out procedure accordingly to Lahiri and Nurnberger [20]. Samples were properly kept labelled and stored at −80 °C until analysis. Only samples with a DNA concentration superior of 20 ng/μL were analyzed.

Subjects were southern Brazil residents, with a majority of subjects with European ethnicity, and a smaller number of individuals with African genetic traits [21], and divided into two groups: sepsis – patients who met sepsis criteria – and controls – critical care patients with no sepsis. Social and demographic data, including age, sex, mortality at the ICU and hospital, SOFA e APACHE II scores were obtained from all patients, in addition to the occurrence septic shock and death. Confidentiality was observed for all samples.

Sepsis was defined as a "clinical syndrome defined by the presence of both documented or suspected infection and a systemic inflammatory response (SIRS)". Signals and symptoms for sepsis were considered as described by Levy and colleagues [22]. Severe sepsis was defined as sepsis accompanied by hypoperfusion or organ dysfunction, while septic shock is defined as severe sepsis that demands sustained use of vasopressive drugs.

Exclusion criteria included human immunodeficiency virus infection; patients in immunosuppressive therapy; non-Caucasian ancestry; pregnant or lactating women. Patients readmitted to the ICU were excluded.

2.2. KIR genotyping

KIR was genotyped using the polymerase chain reaction method with sequence-specific oligonucleotide (PCR-SSO, One Lambda® Inc, California. USA) for 16 KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DP1 and 3DS1). The KIR-SSO Genotyping Test applies Luminex technology (One-Lambda® Inc, Canoga Park, CA, USA) and was performed according to the manufacturer's instructions. Briefly, genomic DNA, specific primers and manufacturer's solutions were mixed and the DNA amplified by the GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, CT, USA). Resulting products were visualized under UV light after electrophoresis in 1% agarose gel containing ethidium bromide. Internal control was included in each PCR reaction.

2.3. HLA genotyping

HLA genotyping was performed using the polymerase chain reaction method with sequence-specific primers (PCR-SSP). HLA typing Cw epitope C1 (Cw 01, 03, 07 {01– 06}, 08, 12 {02, 03, 06}, 14, 16 {01, 03, 04}), and C2 (Cw 02, 04, 05, 06, 0707, 12 {04, 05}, 15, 1602, 17, 18) were performed using PCR-SSP, as described by Jones et al. [17]. HLA-Bw4, A3 and A11 genotyping were also performed using PCR-SSP, accordingly to Bunce et al. [23].

2.4. Ethics

This study was approved by the Research Ethics Board of the Porto Alegre Clinical Hospital (HCPA, CAEE 10555212.6.0000.5327, protocol number 13-0038) and was conducted in accordance with the Declaration of Helsinki. All subjects or their surrogates received detailed explanations and provided written consent prior to inclusion in this investigation.

2.5. Statistical analysis

Comparison of the KIR gene frequency with the control group was executed by Pearson chi-square with continuity correction, and in a few, where the expected difference between the two groups was small, Fisher's exact test was employed. Significance value was calculated using SPSS for Windows version 21.0 (SPSS Inc., Chicago, IL, USA). Results were also analyzed after Bonferroni corrections due to multiple comparisons. A p value ≤ 0.05 after correction was considered statistically significant. We assessed the Hardy–Weinberg equilibrium for KIR3DS1 and KIR3DL1 genotypes (considering the copy number of KIR3DS1) in the groups of subjects by using X^2 test. Linkage Disequilibrium was calculated as per the Lewontin's principle using MASS KIR Analyzer software. The strong positive LD has been assigned to the KIR genes with a LD score ranging between 0.8 and 1 [24].

3. Results

Two hundred and seventy-one patients met inclusion criteria and were divided into sepsis (211 patients diagnosed with sepsis) and control (60 critical care patients with no sepsis) groups. Statistical analysis indicated no differences between groups regarding age and gender. Demographic data are shown in Table 1.