Human Immunology 76 (2015) 742–746

Contents lists available at ScienceDirect

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

FAS −670 A/G polymorphism may be associated with the depletion of CD4$^+$ T lymphocytes in HIV-1 infection

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ARTICLE INFO

Article history:
Received 29 August 2014
Revised 12 August 2015
Accepted 27 September 2015
Available online 30 September 2015

Keywords:
FAS
FASL
HIV
Polymorphism
TCD4$^+$

ABSTRACT

In this study, the polymorphisms in the *FAS* and *FASL* genes was investigated in a sample of 198 HIV-1-seropositive individuals and 191 seronegative controls to evaluate a possible association between polymorphisms and the infection. The identification of the *A* and *G* alleles of the *FAS* −670 polymorphism was accomplished through polymerase chain reaction assays followed by digestion with the restriction enzyme *Mva*I. The identification of the *A* and *G* alleles of the *FAS* −124 polymorphism and the *T* and *delT* alleles of the *FAS* −169 polymorphism were performed using the amplification-created restriction site method followed by restriction fragment length polymorphism reactions. The comparative analysis of allelic and genotypic frequencies between the groups did not reveal any significant differences. However, the quantitative analysis of CD4$^+$ T lymphocytes suggests that the *G* allele of the *FAS* −670 A/G polymorphism can be a protective factor against the depletion of these cells in the course of an HIV-1 infection. Polymorphisms in the *FAS* and *FASL* genes were not associated with the number of CD8$^+$ T lymphocytes or the plasma viral load. Our findings suggest that the *FAS* −670 polymorphism may be associated with apoptosis of CD4$^+$ T lymphocytes after infection by HIV-1.

1. Introduction

AIDS resulting from HIV-1 infection is based on the depletion of CD4$^+$ T lymphocytes, and the apoptosis process has been proposed as the mechanism primarily responsible for this depletion [1–3]. The critical apoptotic mechanism in this situation is mediated by FAS receptor activation by its ligand, FASL [2,4–6].

The FAS protein, also known as APO-1 or CD95, is a transmembrane receptor type I protein belonging to the superfamily of TNF receptors [7,8]. This receptor is present on the surface of cells as a monomeric protein and mediates the apoptotic process by interacting with anti-FAS antibodies or its natural ligand [6]. The FAS ligand (FASL, CD95L, CD178) is a 40 kDa type II transmembrane protein that contains 281 amino acids and, along with its receptor, belongs to the TNF superfamily of proteins [9–11].

The FAS/FASL system is involved in the regulation of the immune response and the process of lymphocyte maturation. Apoptosis mediated by the FAS receptor is a mechanism by which cytotoxic CD8$^+$ T lymphocytes destroy their targets, in addition to the perforins and granzymes pathways [12,13].

Several studies have indicated a role for the FAS/FASL interaction in the apoptosis of CD4$^+$ T lymphocytes during HIV-1 infection [5,14–22]. The analysis of the interaction of this system in patients infected by HIV-1 has demonstrated that, prior to anti-retroviral therapy (ARVT), there is an increased susceptibility to FAS-mediated apoptosis. ARVT significantly diminishes FAS-mediated apoptosis and increases the levels of CD4$^+$ T lymphocytes, showing that this FAS-mediated apoptosis directly relates to the depletion of CD4$^+$ T lymphocytes in HIV-1 infection [14].

Several polymorphisms have already been described in the *FAS* and *FASL* genes and are associated with various diseases [23–35]. Based on this information, studies have examined the influence of existing polymorphisms on HIV-1 infection; however, there

Abbreviations: ACRS, amplification-created restriction site; ARVT, anti-retroviral therapy; bDNA, *branched DNA*; FASL, FAS ligand; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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http://dx.doi.org/10.1016/j.humimm.2015.09.031
0198-8859/

are few published studies, and the results remain controversial [36–38].

This study aimed to investigate the possible association between the *FAS* −670 A>G (rs1800682), *FASL* −124 A>G (rs5030772) and *FASL* −169 T>delT (rs5030645) polymorphisms with the quantification of $CD4^+$ and $CD8^+$ T lymphocytes and plasma viral load in individuals infected with HIV-1.

2. Materials and methods

2.1. Population groups studied

The study sample examined was composed of 198 HIV-1-seropositive individuals, male and female, between 19 and 69 years of age. The patients were seen at the Specialized Reference Center for Infectious and Parasitic Diseases (Unidade de Referência Especializada em Doenças Infecciosas e Parasitárias Especiais – URE-DIPE), Belem, Para, Brazil for $CD4^+$/$CD8^+$ T lymphocyte count and plasma viral load determination, as an integral part of the National Network of the Brazilian Health Ministry. Analyzing the records of these patients revealed that 49 individuals had not received ARVT prior to blood collection.

A group of 191 individuals, male and female, between 18 and 62 years of age, seronegative for HIV-1, HCV, HBV and HTLV-1/2, constituted the control group.

To avoid confounding factors associated with ethnic origin, which could bias the genotypic and allelic frequencies Caucasians, Amerindians and Afro-Brazilian communities were excluded and all participants in both groups were from the same ethnic origin (tri-hybrid) and resided in Belem.

The participants were recruited between July 2007 and January 2009, and written informed consent was obtained prior to inclusion in the research study. This study was approved by the Research Ethics Committee of the University Hospital João de Barros Barreto (Hospital Universitário João de Barros Barreto), protocol n° 2092/05, in accordance with the resolution n° 466/2012 from the National Health Council, which addresses regulatory standards and guidelines for research involving human beings.

2.2. Obtaining samples

Blood samples from both groups were obtained using a vacuum collection system containing K_3-EDTA as an anticoagulant for obtaining plasma and peripheral blood mononuclear cells (PBMC).

After collection, samples were sent to the Laboratory of Virology of the Institute of Biological Sciences, Federal University of Para (Universidade Federal do Pará) for $CD4^+$ and $CD8^+$ T lymphocyte quantification tests and determination of plasma viral load; the samples were stored at −20 °C until further use.

2.3. Plasma viral load quantification

The plasma viral load in HIV-1-seropositive individuals was determined by the *branched DNA* (bDNA) method, using the Versant® HIV-1 RNA 3.0 Assay bDNA kit (Bayer Corporation, MA, USA) with the 340 bDNA Analyzer System (Siemens, Deerfield, IL, USA).

2.4. $CD4^+$ and $CD8^+$ T lymphocyte quantification

The count of helper ($CD3^+$, $CD4^+$) and cytotoxic ($CD3^+$, $CD8^+$) T lymphocytes was conducted 4 h after blood collection by flow cytometry (BD FACSCount™, Becton & Dickinson, San Jose, CA, USA) using the FACSCount™ monitoring kit Reagents according to the standard protocol recommended by the manufacturer (Becton & Dickinson).

2.5. Analysis of polymorphisms

Total DNA extraction from peripheral blood leukocytes was performed using the phenol–chloroform method, which involved cell lysis, protein precipitation, DNA precipitation and hydration. The polymorphisms in the *FAS* and *FASL* genes were identified using polymerase chain reaction (PCR) assays followed by enzyme digestion and restriction fragment length polymorphism (RFLP) analysis.

2.6. Identification of the FAS −670 (rs1800682) polymorphism

The PCR reaction was performed at a final volume of 50 μL containing 500 ng of total DNA extracted, 0.2 μM each dNTP, 5 pmol/μL each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.3) and 1.0 U of Taq DNA polymerase. The primer pair used for amplification was (forward) 5′-CTA CCT AAG AGC TAT CTA CCG TTC-3′ and (reverse) 5′-GGC TGT CCA TGT TGT GGC TGC-3′. The amplification reaction was performed at the following temperature conditions: an initial denaturation at 95 °C for 120 s; 35 cycles of 60 s at 95 °C, 60 s at 58 °C and 60 s at 72 °C; and a final extension of 5 min at 72 °C.

The amplified product (322 bp) was incubated with the restriction endonuclease *Mva*I and cleaved into 221- and 101-bp fragments due to a natural restriction site within all alleles. In the case of the *G* allele, another site is created, and the 221-bp fragment is cleaved into 184- and 37-bp fragments, whereas in the presence of the *A* allele, the 221-bp fragment is not digested [33].

2.7. Identification of the FASL IVS2nt −124 (rs5030772) polymorphism

The identification of the *A* and *G* alleles was conducted using the amplification-created restriction site (ACRS) method. The amplification reaction for the 239 bp *FASL* gene was conducted in a volume of 50 μL containing 500 ng of total DNA extracted, 0.2 μM each dNTP, 5 pmol/μL each primer, 50 mM KCl_2, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3) and 1 U of Taq DNA polymerase. The primer pair for this reaction was (forward) 5′-GCA GTT CAG ACC TAC ATG ATT AG**G** AT-3′ and (reverse) 5′-CCA ATT CTC ACC TGT ACC TTC-3′, and it contains an alteration (bold and underlined) that creates a restriction site for the enzyme *Fok*I in the presence of the *G* allele. For product amplification, the following temperatures were used: initial denaturation at 94 °C for 300 s; 35 cycles of 60 s at 94 °C, 60 s at 55 °C, 60 s at 72 °C; and a final extension for 10 min at 72 °C.

The amplified product was then incubated with the restriction endonuclease *Fok*I at 37 °C for 4 h, which cleaves the *G* allele into 211- and 28-bp fragments, while the allele *A* is not digested and results in a 239-bp fragment [37].

2.8. Identification of the FASL IVS3nt −169 (rs5030645) polymorphism

The detection of the T deletion at position IVS3nt-169 in the third intron of the *FASL* gene was conducted using ACRS. The PCR reaction was performed in a volume of 50 μL containing 500 ng of total DNA extracted, 0.2 μM each dNTP, 5 pmol/μL each primer, 50 mM KCl_2, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3) and 1 U of Taq DNA polymerase. The primer pair for this reaction was (forward) 5′-AGG AAA GGA CTT CAA AGC CTA-3′ and (reverse) 5′-TTG ATG CAT CAC AGA ATT TC**G** TC-3′. The reverse primer contains an alteration (bold and underlined) responsible for creating a restriction site for the enzyme *Hinc*II in the presence of T in