Fas–670 promoter polymorphism is associated to susceptibility, clinical presentation, and survival in adult T cell leukemia

L. Farre,* A. L. Bittencourt,[†] G. Silva-Santos,* A. Almeida,* A. C. Silva,* D. Decanine,*
G. M. Soares,* L. C. Alcantara Jr.,[‡] S. Van Dooren,[§] B. Galvão-Castro,[‡] A. M. Vandamme,[§]
and J. Van Weyenbergh*,[‖,1]

*Laboratórios de *Imunorregulação e Microbiologia (LIMI) and ‡Saúde Pública (LASP) CPqGM, FIOCRUZ, Salvador-BA, Brazil; †Hospital Universitário Professor Edgard Santos (HUPES), Salvador-BA, Brazil; ‖Instituto de Investigação em Imunologia (iii–Millenium Institute), SP, Brazil; and §Clinical and Epidemiological Virology, Rega Institute, Leuven, Belgium*

Abstract: Fas (TNFRSF6/Apo-1/CD95) is a type I transmembrane receptor, which mediates apoptosis. Fas gene mutations, aberrant transcripts, and abundant expression of Fas have been reported in adult T cell leukemia (ATL). To further elucidate the role of Fas in ATL pathogenesis, we investigated whether the –670 *FAS* promoter A/G polymorphism (STAT1-binding site) might contribute to susceptibility and clinical outcome in ATL. Thirty-one patients with ATL, 33 healthy, human T lymphotropic virus type 1-infected individuals, and 70 healthy, uninfected controls were genotyped for the *FAS* –670 polymorphism by PCR-restriction fragment-length polymorphism. The AA genotype was significantly over-represented in ATL patients in comparison with healthy controls ($P=0.006$), as well as asymptomatics ($P=0.037$), corresponding to an odds ratio (OR) of 3.79 [95% confidence intervals (CI; 1.28–11.41)] and 4.58 [95% CI (1.13–20.03)], respectively. The AA group also comprised significantly more aggressive (acute and lymphoma) clinical subtypes [$P=0.012$; OR=8.40; 95% CI (1.60–44.12)]. In addition, we observed a statistically significant association between GG genotype and survival (log rank test, $P=0.032$). Finally, IFN-γ-induced but not basal FAS mRNA levels were increased significantly ($P=0.049$) in PBMCs from AA versus GG individuals, demonstrating the IFN-dependent functionality of the –670 polymorphism. In conclusion, our results demonstrate that a functional Fas promoter polymorphism is significantly associated to susceptibility, clinical manifestation, and survival in ATL. *J. Leukoc. Biol.* 83: 220–222; 2008.

Key Words: *TNFRSF6 · ATL · STAT1 · HTLV-1 · apoptosis*

Adult T cell leukemia (ATL) is an aggressive neoplasm of activated T lymphocytes caused by human T lymphotropic virus type 1 (HTLV-1) infection and usually occurs in 2–4% of asymptomatic carriers after a 20- to 40-year latency period. As a majority of infected individuals remains asymptomatic, host genetic factors are presumed to play a pivotal role in ATL development. Regarding genetic susceptibility to ATL, few data are available. A significant association to the −857T TNF polymorphism has been described [1], whereas P53 codon 72 polymorphism was associated to ATL progression but not to susceptibility [2]. Fas (also known as TNFRSF6, CD95, or APO-1) is a type I transmembrane receptor belonging to the TNFR superfamily, which plays a key role in apoptotic signaling in a variety of cell types. Deregulation of the FAS signaling pathway has been shown to participate in immune escape and tumorogenesis and has been associated with differentiation, invasiveness, and metastasis of cancer cells. Mutations in *FAS* lead to lymphadenopathy and an increase in the incidence of B cell lymphoma [3]. Furthermore, functional polymorphisms in the *FAS* gene are associated with susceptibility to cancer, such as acute myeloid leukemia [4] and cervical carcinoma [5]. *FAS* gene mutations, aberrant transcripts, and deregulation of Fas expression have been reported in ATL cells [6, 7]. To further elucidate the role of Fas in ATL pathogenesis, we investigated whether a single nucleotide polymorphism at position −670 in the *FAS* gene promoter [8] that occurs in a STAT1-binding motif [IFN-γ-activated sequence (GAS)] might contribute to susceptibility and clinical outcome in ATL.

The ethical committee of Hospital Universitário Professor Edgard Santos (Salvador-BA, Brazil) approved this study; informed consent was obtained from all participants. Diagnosis of ATL, HTLV-1 infection, and clinical subtype classification was made according to published criteria [9], combining clinical and anatomo-pathological data, ELISA (Murex, Paris, France), Western blot (INNO-LIA, Innogenetics, Belgium), flow cytometry, and inverse PCR to detect monoclonal integration [10,

11]. Genotyping for the Fas −670 A/G polymorphism by PCR-restriction fragment length polymorphism (RFLP) was performed as described [8]. Briefly, a 331-bp fragment of the FAS promoter was amplified using specific primers (5′-CTAC-CTAAGAGCTATCTACCGTTC-3′ and 5′-GGCTGTCCATGT-TGTGGCTGC-3′) and digested with *Mva*I. We compared the allelic distribution of this polymorphism in 31 patients with ATL, 33 healthy HTLV-1-infected individuals (seropositives), and 70 healthy uninfected controls (HUC; seronegatives), genotyped by PCR-RFLP. With this sample size, we had 80% power to detect significant differences in genotype frequencies at odds ratios (OR) of at least 3.75. Allele frequencies and genotype distributions of *FAS* −670 polymorphism for each group are shown in **Table 1**. The functional AA genotype (corresponding to the consensus STAT1-binding motif) was the most frequent in patients with ATL (38.7%), whereas 35.5% displayed the GG genotype, and 25.8% were heterozygous GA. ATL genotype distribution was significantly different from HUC (χ^2, $P=0.019$) and HIC (χ^2, $P=0.014$). The AA genotype was significantly over-represented in ATL patients in comparison with healthy controls (χ^2, $P=0.006$), as well as asymptomatics (Fisher's exact test, $P=0.037$), corresponding to an OR of 3.79 [95% confidence intervals (CI; 1.28–11.41)] and 4.58 [95% CI (1.13–20.03)], respectively. In addition, neither allelic distribution ($P=0.44$) nor genotype frequencies ($P=0.76$) were significantly different between asymptomatic HTLV-1-infected subjects and healthy controls. Thus, Fas promoter polymorphism does not seem to be associated with risk of infection but more specifically, with the process of leukemogenesis. Next, we examined if the −670 polymorphism was associated to clinical manifestation and outcome in ATL, which is usually classified into four subtypes: acute, lymphoma, chronic, and smoldering, according to published criteria [9]. Chronic and smoldering ATL have a less-aggressive, clinical course, whereas acute and lymphoma subtypes display a decreased survival time. When analyzing clinical subtypes in ATL patients according to Fas promoter genotypes, the AA group comprised significantly more aggressive clinical subtypes (acute and lymphoma) in comparison with GA + GG groups [$P=0.012$; OR=8.40; 95% CI (1.60–44.12)]. We also observed a statistically significant linear tendency (χ^2 for trend, $P=0.006$) for the presence of none, one, or two functional A alleles (GG, AG, and AA, respectively) and manifestation of aggressive clinical subtypes. The AA group comprised

TABLE 1. Genotype Frequencies of FAS–670 Polymorphism in ATL and Control Groups

Genotype	ATL ($n=31$)	HIC[a] ($n=33$)	HUCa ($n=70$)
A/A	12 (38.8%)[b]	4 (12%)	10 (14.3%)
G/A	8 (25.8%)[b]	19 (58%)	31 (44.3%)
G/G	11 (35.4%)[b]	10 (30%)	29 (41.4%)

[a] Healthy HTLV-1-infected controls (HIC). [b] ATL genotype distribution was significantly different from healthy controls (χ^2, $P=0.019$) and asymptomatics (χ^2, $P=0.014$). All three populations were in Hardy-Weinberg equilibrium. Allele and genotype frequencies were not significantly different between healthy HTLV-1-infected subjects and HUC (χ^2, $P=0.44$). $P<0.05$ was considered statistically significant.
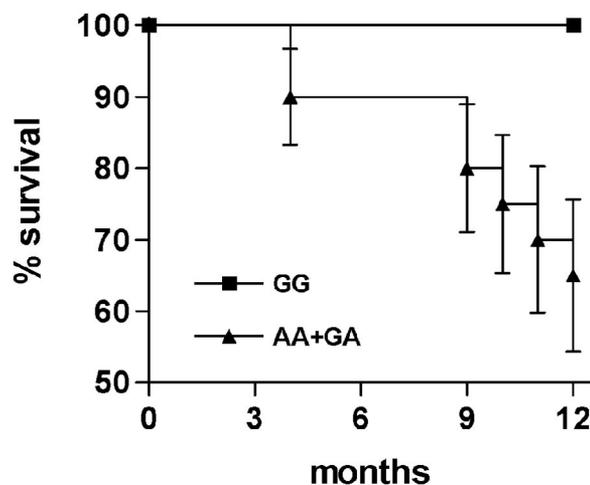

Fig. 1. Patients with AA and GA genotypes were grouped (no significant difference in survival was observed between AA and GA genotypes), and survival rates during the first year of follow-up were compared with the GG group. Onset was calculated from beginning of clinical symptoms; no significant differences in a patient's age at onset were observed between groups. Log rank test was used for survival curves ($P=0.032$).

57.1% of acute patients. Moreover, a lymphoma subtype was observed only in patients with at least one functional A allele [homozygous AA (71.4% of lymphoma forms) and heterozygous GA (28.6% of lymphoma forms)], as none of the lymphoma subtype patients presented a GG genotype. In addition, 63.6% of GG and the 50% of GA patients manifested a smoldering type of ATL, whereas in the AA group, only 25.0% of cases presented a smoldering subtype. Therefore, the AA genotype of the *FAS* −670 polymorphism might not only predispose to ATL development but also to a more aggressive clinical presentation. We also investigated if the −670 polymorphism had any effect on survival in ATL, as determined from onset of disease until death or during at least 1 year of follow-up for patients who were still alive. We observed a statistically significant association between GG genotype and survival (log rank test, $P=0.032$), as shown in **Figure 1**. Thus, the presence of at least one A allele was associated significantly with increased mortality during the first year of follow-up from ATL onset, whereas no significant difference in survival was observed between AA and GA genotypes.

Finally, as the −670 polymorphism corresponds to a STAT1-binding site, we investigated if there was an association between genotype and IFN-induced Fas expression. A previous study [12] has demonstrated lower affinity STAT1 binding to the GG variant, as compared with the AA consensus GAS. It is surprising that although conflicting results were obtained regarding basal transcription in AA versus GG genotypes [12, 13], no data are available on IFN-induced Fas expression in different genotypes. Therefore, we have compared basal and IFN-γ-induced Fas mRNA levels in PBMCs from 12 healthy donors (four GG, five GA, and three AA genotypes). As shown in **Figure 2**, IFN-γ was unable to induce Fas mRNA levels (0.6±2.0% increase) in GG individuals, but a gradual increase was observed with each functional A allele: 8± percent in GA and 19.2± percent in AA individuals ($P=0.049$, as compared with GG, t-test). However, no differences in basal FAS mRNA levels were observed between
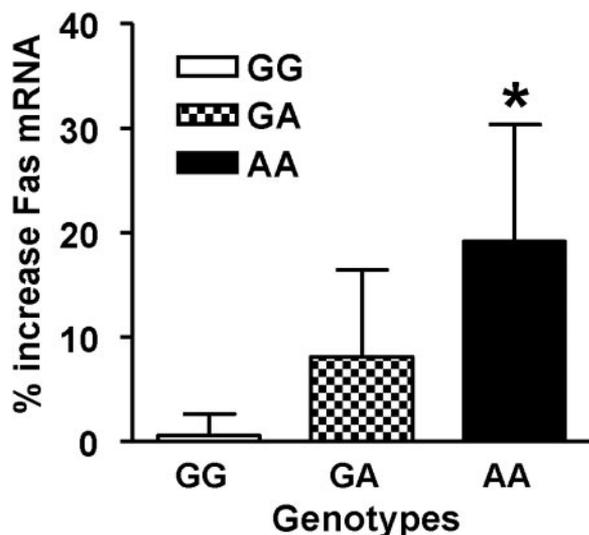
Fig. 2. PBMCs from healthy controls (three AA, five GA, and four GG) were cultured for 12 h in the absence or presence of 1000 IU/ml IFN-γ. Total RNA was extracted, reverse-transcribed, and amplified using specific primers for β-2-microglobulin (CATCCAGCGTACTCCAAAGA and GACAAGTCT-GAATGCTCCAC) and FAS (TCCTACCTCTGGTTCTTACG and AGATCTG-GATCCTTCCTCTT), followed by PAGE and quantification using ImageJ software. Fas mRNA levels were normalized to β-2-microglobulin levels; IFN-γ induction of FAS mRNA is represented as percent increase over unstimulated cells. One-tailed t-test of log-transformed values was used to compare the effect of IFN-γ between genotypes (*, $P=0.049$ for AA vs. GG).

genotypes (not shown), indicating that the −670 polymorphism determines IFN-induced and hence, STAT1-mediated FAS expression rather than interfering with basal promoter activity. We hypothesize that increased Fas expression (functional AA and/or GA genotypes) might contribute to ATL susceptibility and clinical progression by affecting normal host cells involved in tumor surveillance, in agreement with recent observations in cervical cancer, where genetic polymorphisms in the Fas-Fas ligand pathway might confer susceptibility through enhanced activation-induced cell death of tumor-specific T cells [14]. Leukemogenesis in ATL is expected to be a multifactorial process as a result of several genetic and epigenetic changes described affecting cell cycle and apoptosis regulatory genes, generally requiring several decades [15]. Furthermore, additional apoptosis-independent functions of Fas have been described [16], including induction of proliferation in T cells, through which Fas might contribute to ATL pathogenesis.

Studying genetic polymorphisms not only reveals potential players in oncogenesis but also has important clinical significance, because of its association to morbidity and mortality. Genotyping seropositive individuals for −670 FAS polymorphism might lead a more careful follow-up and earlier detection of ATL cases, considering that a single polymorphism roughly doubles the risk of developing ATL (AA vs. GA+GG). In addition, AA genotype almost triples the risk of an aggressive form of ATL, which is typically resistant to chemotherapy but might respond to IFN-α + AZT, whereas milder forms mostly remain untreated. Confirmation of our results in other ATL endemic areas will be necessary before Fas genotyping might enter into practice in ATL clinical management. In conclusion, our results demonstrate that a functional Fas promoter polymorphism is significantly associated to susceptibility, clinical manifestation, and survival in ATL.

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