Cytokine 111 (2018) 265–271

Contents lists available at ScienceDirect

Cytokine

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

IFNG +874 A/T is associated with acute lymphoblastic leukemia in Mexican Mestizos

Betsy Annel González-Quezada[a,b], Hilario Flores-Aguilar[a,b], Alberto Olaya-Vargas[c], Haydee Salazar-Rosales[c], Martín Pérez-García[c,1], Luis Manuel Valero-Saldaña[d], Brenda Lizeth Acosta-Maldonado[d], Roberto Ovilla-Martínez[e], Clara Gorodezky[a,b,*]

[a] Department of Immunology and Immunogenetics, InDRE, Secretary of Health, Francisco P. Miranda #177, col. Lomas de Plateros, Del. Álvaro Obregón, CP 01480 Mexico City, Mexico
[b] Fundación Comparte Vida, A.C. Galileo #92, col. Polanco, Del. Miguel Hidalgo, CP 11550 Mexico City, Mexico
[c] Instituto Nacional de Pediatría, Secretary of Health, Insurgentes Sur #3700, Letra C, Delegación Coyoacán, CP. 04530 Mexico City, Mexico
[d] Instituto Nacional de Cancerología, Secretary of Health, Av. San Fernando #22, Col. Sección 16, C.P. 14080 Mexico City, Mexico
[e] Hospital Ángeles Lomas, Hacienda de las Palmas, Col. Valle de las Palmas, CP 52763 State of Mexico, Mexico

ARTICLE INFO

Keywords:
IFNG +874 T/A
IL2 −330 G/T
rs2430561
rs2069762
Acute lymphoblastic leukemia
Mexican Mestizos

ABSTRACT

Acute lymphoblastic leukemia (ALL), the most common type of cancer in children worldwide, has one of the highest incidence rates in Mexico. It is a multifactorial disease and different cytokine single nucleotide polymorphisms (SNP), have been associated with ALL expression. Few studies have been published analyzing *IFNG* +874 T/A and *IL2* −330 G/T in this type of leukemia. These SNPs are involved in high or low expression, and are central to cellular immunity, influencing greatly tumor growth. The purpose of this work was to explore the association of *IFNG* +874 A/T (rs2430561) and *IL2* −330 G/T (rs2069762) SNPs with ALL susceptibility and/or protection in 488 Mexican Mestizos patients, as compared to 950 Mexican Mestizo healthy controls. The results demonstrated that *IFNG* +874 T allele (pc = 0.00004, OR = 0.673) and the TT genotype (pc = 0.00015, OR = 0.349), protect against ALL expression with no specific gender association; however, the TT homozygote genotype (vs. TA + AA) seems more protective in males (pc = 0.00683). *IL2* −330 G/T does not contribute to the development of ALL. In healthy Mexicans, the most common genotypes for *IL2* and *IFNG*, are the low cytokine producers, suggesting that the genetic background in this ethnic group, may be partly responsible for the high incidence of ALL. These results show for the first time in Mexicans, the relevant role that *IFNG* SNP has in the genetic etiology of ALL. Thus, a large group of patients belonging to different ethnicities will be very helpful to study in order to demonstrate if these SNPs contribute to the genetic etiology of ALL, as shown here in Mexican Mestizos.

1. Introduction

Cancer is an important health problem worldwide; only in the USA, 442.7 new cases/100,000 are diagnosed every year with a mortality rate of 166 deaths/100,000/year [1]. The incidence of different neoplasms are influenced by age; while breast, lung and prostate cancer are most common in adults, leukemia is the most common one in children [2–5]. The incidence of leukemia varies with gender, being higher in males [2,3]. The disease has different rates of cases/million according to the ethnicity; for example, between Caucasians from the east (39.3) and those of western of Europe (45.7) [3] or between Non-Hispanic blacks (29.9), Whites (46.9) or Hispanics (59.6) from the USA [2]. Mexican Mestizos from Mexico City own one of the highest incidence rate of leukemia in the world with 57.6 [6]. Among all types of leukemia, the most common subtype in children under 15 years is acute lymphoblastic leukemia (ALL) accounting for around 80% of all cases [7]. The ALL incidence fluctuates between 18 and 35 cases/million in children, being higher in male Hispanics living in the USA, with an

* Corresponding author at: Department of Immunology and Immunogenetics, InDRE, Secretary of Health, Francisco P. Miranda 177, Laboratory Building, 2nd Floor, Col. Lomas de Plateros, Mexico City 01480, CDMX, Mexico.
E-mail addresses: bgonzalez@fundacioncompartevida.org.mx (B.A. González-Quezada), hflores@fundacioncompartevida.org.mx, hilario.flores@salud.gob.mx (H. Flores-Aguilar), clarag@unam.mx, cgorodea@fundacioncompartevida.org.mx, clara.gorodezky@salud.org.mx (C. Gorodezky).
[1] Present address: Hospital Infantil Teletón de Oncología – HITO, Anillo vial II Fray Junípero Serra 1999, Rancho Menchaca, 76140 Santiago de Querétaro, Qro, Mexico.

https://doi.org/10.1016/j.cyto.2018.08.014
Received 26 April 2018; Received in revised form 10 August 2018; Accepted 13 August 2018

incidence over 40 cases/million [2]. It is relevant to emphasize that Mexico City has also, one of the highest incidences of ALL in the world, with 49.5 cases/million (2006–2007) [6] with higher mortality rates as compared with other countries [7,8]. The reasons for these differences are not fully understood, however it is well known that both, environmental factors like early exposure to infections or to other aggressors, housing lifestyle, ionizing radiation among others, which interact with genetic factors such as genetic syndromes, mutations, gene rearrangements, or different genetic polymorphisms, influence the expression of ALL [2,9,10]. It has also been suggested that a high prevalence exists in Mexico of gene rearrangements associated with the etiology or with a poor prognosis in ALL in children [7]. Different genes have been associated with susceptibility in ALL, through GWAS reports, among them, *AT-rich interactive domain 5B* (*ARID5B*) gene, *Ikaros family zinc finger protein 1* (*IKZF1*) gene [11–15], *CCAAT/enhancer-binding protein epsilon* (*CEBPE*) [11,13–16], *cyclin-dependent kinases* (*CDKN2A/B*) [14,15,17–20], *phosphatidylinositol-5-phosphate 4-kinase type 2 alpha* gene (*PIP4K2A*) [13], *phospholysine phosphohistidine inorganic pyrophosphate phosphatase* (*LHPP*) and *ETS-domain protein* (*ELK3*) [18]. Other studies analyzing candidate genes have shown different associations with ALL, such as the AA genotype of killer cell immunoglobulin-like receptors (*KIR*) genes [21], *HLA-DPB1* of the Major Histocompatibility Complex [22,23], heat shock protein genes (*HSPA1B*) [24], cytokine SNPs polymorphisms or differences in its expression [9,23,25,26]. It is well documented that cytokines and their genes are involved in the immunopathogenesis of infections, autoimmune and malignant diseases, such as ALL [9,27,28].

ALL patients show a defective immune system with a lower population of interleukin 2 (IL-2) and interferon gamma (IFN-γ) producer cells such as Th1 and Tc1 cells [25]. IL-2 is a T-cell growth factor, produced mainly by activated $CD4^+$ T cells, that has the ability of stimulating the growth of $CD8^+$ T cells, and is responsible of the differentiation and homeostasis of $CD4^+$ and $CD8^+$ T cells [29,30]. IL-2 is a pleiotropic cytokine that also possesses antitumor properties, therefore, high doses of this cytokine is used as an immunotherapeutic agent in different neoplasms with good results of evidence of cancer regression [29,31]. IFN-γ, a Th1 pro-inflammatory cytokine, is mainly produced by activated CD4+ T cells, cytotoxic CD8+ cells and activated Natural Killer (NK) cells [32,33]. It also plays a relevant role in the enhancement of the Th1-immune response through the modulation, activation and differentiation of T cells. IFN-γ has also been pointed out as an antitumor agent by inhibiting proliferation, metabolism, angiogenesis, and stimulating apoptosis of cancer cells [32,33].

Since both IL-2 and IFN-γ cytokines, have anti-proliferative and anti-tumoral properties [29,34,35], a higher expression of any of them confers protection to tumor growth [25]. High or low cytokine protein production are associated with inherited SNPs of these cytokines [36,37]. It has been shown for *IFNG*, that a single transition of adenine to thymine on the first intron on *IFNG* +874 (rs2430561) modifies its cytokine production, since *IFNG* +874 T matches with the transcription factor NF-κB binding site, which enhances *IFNG* transcription and thus individuals carrying *IFNG* +874TT genotype produce higher levels of IFN-γ than individuals with TA and AA genotypes [37]. Previous reports have demonstrated susceptibility/protective effects of *IFNG* +874 T/A SNP on viral infections [38], autoimmune diseases [39], different types of cancer in Asian and Caucasian populations mainly [25,27,28,32,33,40–44] and chronic lymphocytic leukemia (CLL) but not for ALL [45] or T-cell large granular lymphocyte leukemia (T-LGL) [41].

IL2 G > T polymorphism located in a promoter region at −330 produces different levels of the cytokine according to the genotype. *IL2* −330 GG produces a threefold increase of its protein expression as compared to *IL2* −330 TT or GT [36]. Three *meta*-analysis in Asians and Caucasians for different neoplasms, showed a higher risk in *IL2* −330 G patients [46,47], with a specific association with lymphoma [47]. The third one, failed to find any association with *IL2* −330 G > T [48].

Table 1
Demographics of Mexican Mestizo ALL Patients and Controls.

Group	Mean age at diagnosis (range)	Gender		Total (%)
		Male (%)	Female (%)	
ALL patients				
Children	9.6 (1–18)	184 (59%)	126 (41%)	310 (63.5%)
Adults	30.8 (19–65)	112 (63%)	66 (37%)	178 (36.5%)
Total	17.3 (1–65)	296 (61%)	192 (39%)	488 (100%)
Healthy controls				
Adults	33.9 (18–65)	349 (37%)	601 (63%)	950 (100%)

Despite of the several studies of *IFNG* +874 and *IL2* −330 in different malignancies and in diverse populations, the results remain controversial. For this reason and because, to our knowledge, no similar study has been published in Mexican Mestizo patients with ALL, we conducted the present analysis. The intent was to ascertain if *IFNG* +874 A/T (rs2430561) and *IL2* −330 G/T (rs2069762) SNPs encoding mediators of anti-tumoral cytokines SNPs which are involved in determining the level of the cellular immune response, are associated with ALL and if they correlate with gender, in a large sample size of Mexican Mestizo patients born and living in Mexico.

2. Material and methods

2.1. Subjects

The demographics are depicted in Table 1. All subjects were Mexican Mestizos, born and living in Mexico and signed an informed consent according to the Declaration of Helsinki [49].

2.1.1. Patients

A total of 488 patients diagnosed with acute lymphoblastic leukemia were recruited between 2000 and 2014. The pediatric patients were treated at the Instituto Nacional de Pediatría; the adult patients were diagnosed either at The Instituto Nacional de Cancerología or at the Hospital Ángeles de las Lomas. Diagnosis of ALL was based on the presence of anemia related symptoms, thrombocytopenia and neutropenia with demonstration of ≥20% positive bone marrow lymphoblasts in bone marrow aspirate and biopsy. The inmunophenotypes were evaluated by flow cytometry and genetic characterization was done through karyotypic characterization and Interphase Fluorescence *In Situ* Hybridization (FISH). BCR-ABL, MLL rearrangements and RT-PCR were performed to identify chromosome alterations. Lumbar puncture was done at diagnosis [50].

2.1.2. Controls

The group consisted of 950 Mexican Mestizo healthy donors recruited from the **DONORMO**-The Mexican Unrelated Donors Registry. None of the donors reported personal or family antecedents of cancer or any chronic infectious, or degenerative diseases.

2.2. DNA extraction and genotyping

DNA was extracted from peripheral blood with the Maxwell16 instrument (Promega Corporation, Madison, WI, USA) according to the technical manual. Genotyping of *IL2*-330 G/T (rs2069762) was performed using a Taqman probe (C_15859930_10, part#4351379, Thermo Fisher Scientific, Waltham, MA, USA); for *IFNG* +874 A/T (rs2430561) primers and Taqman probes were synthesized by Thermo Fisher Scientific as described previously [51]. Assays were run on an ABI 7500 Real Time PCR instrument, according to the previously described methodology [51].