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Interleukin-10 promoter gene polymorphisms have no clear influence on interleukin-10 protein secretion in AIDS-associated B-cell lines

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Abstract

Interleukin-10 (IL-10) is a pleiotropic cytokine involved in several immune responses and expressed by a variety of cell types. IL-10 interacts with at least two subunits of the IL-10 receptors (IL-10R1 and IL-10R2), which are members of the interferon receptor family, and play important roles in ligand binding and signaling. Using reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) methods, the mRNA expression and secretion patterns of IL-10 were studied. IL-10R1 and IL-10R2 mRNA expression patterns were also studied in the tumor-derived human B-cell lines. IL-10 protein is expressed and predominantly secreted by AIDS-associated B-cell lines (AABCL). However, IL-10R1 and IL-10R2 are constitutively and ubiquitously expressed in all the B-cell lines included in our study. These results suggest that B-cell IL-10 functions as an autocrine growth factor, in AABCL. Furthermore, we report that higher secretion of IL-10 observed in AABCL could be due to the specific GCC haplotype of IL-10 promoter polymorphisms, although no specific correlation was observed between IL-10 promoter polymorphisms and IL-10 protein secretion as analyzed by PCR-sequence specific primers methodology and ELISA, respectively.

Keywords: AIDS; B-cell lines; EBV; Interleukin-10; PCR-SSP

IL-10 is a pleiotropic cytokine that has diverse effects in regulating most lymphoid and myeloid cells. IL-10 is produced by a variety of cells, including activated monocytes, B-cells, T-cells, keratinocytes, thymocytes, and macrophages [1,2]. IL-10 is an immunomodulatory cytokine involved in inflammatory responses and pathology of autoimmune disease [3]. In addition, IL-10 regulates the proliferation and differentiation of B-cells. The biological effects of IL-10 are orchestrated through the IL-10 receptor complex, which is composed of two subunits, IL-10 receptor 1 (IL-10R1) and IL-10R2. Both subunits belong to the class II cytokine receptor family (CRF2), which also contains the receptor for interferon (IFN)-α and IFN-γ [3].

The IL-10 gene has been mapped to chromosome 1q31-32 and is encoded by five exons [3]. In the IL-10 promoter region, at least 23 single nucleotide polymorphisms (SNPs) and two microsatellites, named IL-10.R and IL-10.G, have been reported [4]. The most investigated SNPs are −1082 G/A, −819 C/T, and −592 C/A upstream from the transcription start site [5]. These three dimorphisms exhibit strong linkage disequilibrium, especially the −819 and −592 polymorphisms [4,5]. In Caucasians, SNPs appear in three preference potential haplotypes GCC, ACC, and ATA and in the extremely rare GTA [4,5]. It is also reported that −1082 G/A appears within a putative *ETS*-factor binding site, −819 C/T lies within a positive regulatory site, and −592 C/A may be a STAT3 binding site and a negative regulatory region [6,7]. Many reports suggest that IL-10 SNPs may be associated with or enhance the risk of diseases such as systemic lupus erythmatosus (SLE)

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[7], Epstein–Barr virus (EBV)-associated gastric carcinoma [8], ovarian cancer [9], sudden infant death syndrome (SIDS) [5], and many more.

IL-10 exhibits strong sequence homology to B-cell receptor factor-1 (BCRF1), an open reading frame in the EBV genome [10]. EBV can directly cause a polyclonal B-lymphoid disease in immunodeficiency patients. Moreover, it is also an etiologic agent in monoclonal B-lymphoid diseases such as Burkitt's lymphoma (BL), Hodgkin's disease (HD), unusual T-cell lymphomas, and naso-pharyngeal carcinoma (NPC) [11]. BCRF1 encodes viral IL-10 (vIL-10) [3] and the amino acid sequences between mature human IL-10 and EBV vIL-10 are 84% identical [10]. Reports from Helminen et al. [12] suggest that the IL-10 ATA haplotype protects against primary EBV infection by increasing IL-10 levels. IL-10 SNPs may also play a role in development of aggressive non-Hodgkin's lymphoma (NHL), particularly those phenotypes that are associated with low IL-10 production [13]. However, a report from Munro et al. [14] found little evidence between IL-10 SNPs and predisposition to the development of HD.

Previously, Benjamin et al. [1,15] reported copious secretion of IL-10 protein in B-cell lines derived from AABCL, suggesting the significant role played by HIV-1 on IL-10 protein secretion. According to Shin et al. [16], individuals carrying IL-10 −592A allele may be at increased risk for HIV infection. The authors further suggest that influence of IL-10 −592A SNP down-regulates IL-10 production, thereby facilitating HIV-1 replication in vivo and accelerating the onset of AIDS [16]. Employing PCR-sequence specific primers (PCR-SSP) technique, which is a dynamic process requiring highly controlled conditions to ensure discriminatory amplification, we extend the previous observations of IL-10 expression and secretion in tumor-derived B-cells by specific correlation to their genotype/haplotype to IL-10 promoter polymorphism. By such an analysis, we report here the influence of the GCC haplotype on increased secretion of IL-10 protein as observed in AABCL.

Materials and methods

Cell lines and culturing conditions. The 14 tumor B-cell lines included in this study were derived from patients with undifferentiated lymphomas of Burkitt's and non-Burkitt's origin as previously described [17,18]. Table 1 lists the origin and characteristics of the B-cell lines used in this study. The tumor B-cell lines were classified as AABCL or non-AABCL. The five AABCL include IOC-9, 2F7, HBL-1, HBL-2, and HBL-3. IOC-9 was established from an AIDS patient with NHL. 2F7 was established from an AIDS patient with BL. They were purchased from ATCC, Rockville, MD. HBL-1, HBL-2, and HBL-3 were established from patients with AIDS-associated small non-cleaved B-cell lymphomas. They were a kind gift from Dr. Riccardo Dalla-Favera, Columbia University, NY [19]. The HBL-1, IOC-9, and 2F7 cell lines are EBV-positive (EBV^+); while the HBL-2 and HBL-3 cell lines are EBV-negative (EBV^-). No HIV-1 viral sequences were detected in any of these cell lines [19]. The non-AABCL were comprised of eight cell lines of American or African Burkitt's origin and varying EBV status. The five EBV^-, American BL cell lines included BJAB, EW36, CA46, ST486, and MC116. B958 was BJAB superinfected with EBV and was a kind gift from Dr. Michael Steinitz, Hebrew University, Hadassah Medical School, Jerusalem, Israel. The three EBV^+, African BL cell lines included Raji, Daudi, and Namalva. All these cell lines were purchased from ATCC, Rockville, MD.

The cell lines were cultured in RPMI-1640 media (Cellgro) that were supplemented with 10 mM Hepes buffer, 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Fisher Scientific, Atlanta, GA). The cell cultures were grown in a humidified incubator at 37 °C with 5% CO_2 and subcultured every 3–5 days as needed.

Exposure of cells to phorbol 12-myristate 13-acetate. Cells obtained on the fourth day after subcultures were resuspended in fresh medium at a cell density of 1×10^6 cells/ml. Each cell line was divided into two T-25 flasks; one remained as a control, while the second was stimulated

Table 1
Characteristics of B-cell lines

	Cell line	EBV status	Continent	Histology	Tissue source
American Burkitt's lymphoma					
AABCL	IOC9	Positive	N. America	Burkitt's	Peripheral Blood
	2F7	Positive	N. America	Burkitt's	Peripheral blood
	HBL-1	Positive	N. America	UL	Peripheral blood
	HBL-2	Negative	N. America	UL	Pleural effusion
	HBL-3	Negative	N. America	UL	Liver
Non-AABCL	EW36	Negative	N. America	UL	Pleural effusion
	CA46	Negative	S. America	Burkitt's	Ascitic fluid
	ST486	Negative	N. America	UL	Ascitic fluid
	MC116	Negative	N. America	UL	Pleural effusion
	BJAB	Negative	N. America	Burkitt's-like	Lymphoblastoid
	B958	Positive	N. America	Burkitt's-like	Lymphoblastoid
African Burkitt's lymphoma					
Non-AABCL	Raji	Positive	Africa	Burkitt's	Jaw tumor
	Daudi	Positive	Africa	Burkitt's	Orbital tumor
	Namalva	Positive	Africa	Burkitt's	Lymphoblastoid

AABCL, acquired immunodeficiency syndrome (AIDS)-associated B-cell line; EBV, Epstein–Barr virus; UL, undifferentiated lymphoma.