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Expression of human leukocyte antigen-G in systemic lupus erythematosus

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Summary The purpose of this study was to examine the expression of human leukocyte antigen-G (HLA-G) in patients with systemic lupus erythematosus (SLE) and its relation with interleukin-10 (IL-10) production. The study included 50 female SLE patients and 59 healthy female donors. HLA-G expression in peripheral blood and cutaneous biopsies was determined by flow cytometry and immunohistochemistry, respectively. Soluble HLA-G (sHLA-G) and IL-10 were quantified in serum samples by enzyme-linked immunosorbent assay. SLE patients presented with serum sHLA-G and IL-10 levels significantly higher than that observed in controls (median [interquartile range (IQR)] = 43.6 U/ml [23.2–150.2] vs 26.84 U/ml [6.0–45.2], $p = 0.004$; and 1.4 pg/ml [0–2.3] vs 0 pg/ml [0–1.5], $p = 0.01$, respectively). But no correlation was observed between sHLA-G and both IL-10 levels and the disease activity index for SLE patients. The expression of membrane HLA-G in peripheral lymphocytes from SLE patients was low, but higher than in controls (median [IQR] = 1.5% [0.6–1.8] and 0.3% [0.2–0.8], respectively; $p = 0.02$). Finally, these findings were in accordance with the weak expression of HLA-G in skin biopsies. Despite the fact that patients present higher levels of HLA-G than healthy controls, which suggests a possible relevance of this molecule in SLE, it seems not to be related to IL-10 production or disease activity.

Introduction

Human leukocyte antigen-G (HLA-G) is a nonclassic major histocompatibility complex antigen. HLA-G gene differs from other classic HLA class I molecules because of its limited polymorphism, the restricted tissue distribution, and the translation of alternative spliced transcripts that encode seven distinct isoforms: four as membrane bound (G1, G2, G3, and G4) and three as soluble proteins (G5, G6, and G7) [1]. The selective HLA-G mRNA expression pattern in tissues suggests a tight transcriptional control

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ABBREVIATIONS

B-CLL	B-chronic lymphocytic leukemia
ELISA	enzyme-linked immunosorbent assay
HLA-G	human leukocyte antigen-G
IFN-γ	interferon-γ
IL-6	interleukin-6
IL-10	interleukin-10
mAbs	monoclonal antibodies
PBMCs	peripheral blood mononuclear cells
sHLA-G	soluble HLA-G
SLE	systemic lupus erythematosus
SLEDAI	disease activity index for systemic lupus erythematosus patients
TNF-α	tumor necrosis factor-α

of gene expression [2], possibly attributable to the HLA-G promoter region, which significantly differs from that of other HLA class I genes. Cytokines, such as interferons (IFNs) and interleukin-10 (IL-10), induced the expression of this antigen [3,4].

Physiologic HLA-G expression, in nonpathologic situations, is tissue restricted to extravillous cytotrophoblast [5], thymic epithelial cells [6], and cornea [7]. Under pathologic conditions, HLA-G expression has been documented in nonrejected allograft transplants [8,9], in tumors [10], in the course of viral infection [11,12], and during inflammatory diseases (e.g., skin and muscle inflammations, multiple sclerosis) [13–16]. Several studies point toward immunoregulatory functions of HLA-G, especially at the maternal–fetal interface during pregnancy. HLA-G is able to inhibit natural killer-cell-mediated lysis [17,18] and their transendothelial migration properties [19], allogenic T-cell cytotoxicity [20], T-cell proliferation [21,22], and maturation of myelomonocytic cells into functional antigen-presenting cells [23]. In addition to all these cellular effects, HLA-G may act as an immunomodulatory molecule by promoting an immune deviation from Th1 to Th2 response [24–26]. Apart from the immunotolerant role of HLA-G antigens in maternal–fetal tolerance, the acceptance of grafts, and probably the dissemination of tumors, it has been recently proposed that they might play a protective role in inflammatory diseases [27].

The expression of HLA-G is up-regulated by the anti-inflammatory cytokine IL-10 [4], which conversely down-modulates the expression of HLA class I and II molecules. IL-10 is an immunoregulatory cytokine produced by numerous cell types (monocytes [28], T and B cells [29], Kupffer cells [30], keratinocytes [31], and cytotrophoblast cells [32]). This pleiotropic cytokine plays an important role in the regulation of the immune response, mainly by the inhibition of proinflammatory cytokine expression [28], the alteration of antigen presentation on T-cell activation pathways [29,33], and the stimulation of the proliferation and differentiation of B cells [34,35]. Both HLA-G and IL-10 seem to be involved in immune modulation and possibly in induction of immune tolerance. Furthermore, it has been suggested that IL-10 inhibits cellular responses through expression of HLA-G [4,24].

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by B-lymphocyte hyperactivity accompanied by changes in the immunoglobulin repertoire, leading to an increased production of autoantibodies and impaired cell-mediated immunity, which results from both T lymphocytes and antigen-presenting cells. Patients with SLE spontaneously produce high levels of IL-10 that correlate with parameters of disease activity [36,37]. Moreover, continuous administration of neutralizing anti-IL-10 antibodies delays the onset of autoimmunity in a murine model of SLE [38] and improves cutaneous lesions and joint symptoms in patients [39], suggesting a role for IL-10 in the pathophysiology of SLE.

Because SLE is characterized by the secretion of IL-10, which is able to upregulate HLA-G expression, we asked whether HLA-G might be expressed in this disease. The aim of the present study was to investigate the presence of HLA-G molecules in lymphocytes, serum, and skin from SLE patients.

Subjects and methods

Patients and controls

A total of 50 female SLE patients from Hospital Universitario Puerta de Hierro, Madrid (Spain), were included in the study. The inclusion criterion was the fulfillment of four or more of the revised American College of Rheumatology classification criteria [40]. The median age was 46 (range: 19-64). At the time of the study 14% of patients were receiving no treatment; 17% antimalarials (chloroquine or hydroxychloroquine); 35% antimalarials and low-dose steroids ($\leq$15 mg prednisone/day); 24% immunosuppressive agents (mycophenolate mofetil, cyclosporine A, or azathioprine) in combination with antimalarials or low-dose steroids; and 10% antimalarials and high-dose steroids ($\geq$30 mg prednisone/day). SLE activity was assessed by the disease activity index for lupus patients (SLEDAI), described by Bombardier *et al*. [41]. Eighty percent of patients had active disease (>0 at the SLEDAI score, median = 4, range [1–16]), whereas 20% had inactive lupus. Fifty-nine nonselected and nonrelated healthy females without any autoimmune history and 28 female patients with B-chronic lymphocytic leukemia (B-CLL) were used as controls.

Cell separation

After informed consent was obtained, heparinized whole blood was collected from patients and healthy subjects. Peripheral blood mononuclear cells (PBMCs) were then isolated by density gradient centrifugation.

Cell line culture

The choriocarcinoma cell line JEG-3 was cultured in minimum essential medium (Eagle's) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, and 100 U/ml penicillin, and 100 μg/ml streptomycin and was maintained at 37°C in a humidified 5% CO_2 atmosphere. This line was used as a positive control of surface HLA-G expression.

Antibodies

The following antihuman monoclonal antibodies (mAbs) were used in flow cytometry studies: anti-HLA-G-fluorescein isothiocyanate (MEM-G/9, clone specific for the intact β2m-associated HLA-G, this monoclonal antibody detects soluble and membrane isoforms of