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Original research article

HLA-G and anti-HCV in patients on the waiting list for kidney transplantation

Linda Sommese[a,*], Rossella Paolillo[b], Francesco Cacciatore[c], Vincenzo Grimaldi[b], Chiara Sabia[b], Antonella Esposito[b], Antonio Sorriento[a], Carmela Iannone[b], Nicolò Rupealta[d], Gerardo Sarno[e], Michele Santangelo[d], Paride De Rosa[e], Gianfranco Nicoletti[f], Claudio Napoli[g,h]

[a] *U.O.C. Division of Clinical Immunology, Immunohematology, Transfusion Medicine and Transplant Immunology, Regional Reference Laboratory of Transplant Immunology, Azienda Ospedaliera Universitaria (AOU), Department of Experimental Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Napoli, Italy*
[b] *U.O.C. Division of Clinical Immunology, Immunohematology, Transfusion Medicine and Transplant Immunology, Regional Reference Laboratory of Transplant Immunology, Department of Internal and Specialty Medicine, Azienda Ospedaliera Universitaria (AOU), Università degli Studi della Campania "Luigi Vanvitelli", Napoli, Italy*
[c] *IRCCS Salvatore Maugeri Foundation, Scientific Institute of Telese, Benevento, Italy*
[d] *University Federico II, Napoli, Italy*
[e] *San Giovanni di Dio e Ruggi D'Aragona, Università Ospedaliera, Salerno, Italy*
[f] *Multidisciplinary Department of Medical-Surgical and Dental Specialties, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*
[g] *Department of Medical, Surgical, Neurological, Metabolic and Geriatric Sciences, U.O.C. Division of Clinical Immunology, Immunohematology, Transfusion Medicine and Transplant Immunology, Regional Reference Laboratory of Transplant Immunology, Azienda Ospedaliera Universitaria (AOU), Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy*
[h] *IRCCS SDN, Naples, Italy*

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ABSTRACT

Purpose: Human leukocyte antigen (HLA)-G is a non-classic major histocompatibility complex HLA class I molecule. HLA-G may have tolerogenic properties which are linked to epigenetic-sensitive pathways. There is a correlation of sHLA-G levels and graft acceptance in transplantation studies. There are previous data on correlation of sHLA-G with graft rejection as well as with viral infections such as hepatitis C virus (HCV) in kidney transplanted patients. Here, we report the sHLA-G expression in patients on the waiting list for kidney transplantation, with and without anti-HCV compared to a control group.

Methods: Serum of 67 patients on the waiting list for kidney transplantation (n = 43 with anti-HCV and n = 24 without anti-HCV) was analyzed. Among these patients, n = 39 were on the waiting list for the first transplantation, while n = 28 were patients who returned in the list. The control group included n = 23 blood donors with anti-HCV (n = 13) and without anti-HCV (n = 10).

Results: The expression of sHLA-G was significantly lower in the control group (39.6 ± 34.1 U/ml) compared to both - patients on the waiting list for the first transplantation (62.5 ± 42.4 U/ml, $p = 0.031$) and patients who returned in the list (76.7 ± 53.9 U/ml, $p = 0.006$). No significant differences were observed in all anti-HCV positive groups. A positive linear correlation between sHLA-G and TNF-α, and patient age was observed.

Conclusions: Serum sHLA-G values were significantly increased in both - patients on the waiting list for the first transplantation and patients who returned in the list, as compared to control group. Our findings confirm the key tolerogenic role of sHLA-G levels as epigenetic-related marker for measuring the state of kidney allograft acceptance.

1. Introduction

The human leukocyte antigen-G (HLA-G) got much attention due to its multiple functions in the immune system as well as into epigenetic platform [1–6]. It plays a fundamental role in inducing tolerance by its immunosuppressive effects on all types of immune cells [7,8].

* Corresponding author at: Department of Experimental Medicine, U.O.C. Division of Clinical Immunology, Immunohematology, Transfusion Medicine and Transplant Immunology, Regional Reference Laboratory of Transplant Immunology, Azienda Ospedaliera Universitaria, Università degli Studi della Campania "Luigi Vanvitelli" Piazza Miraglia, 2 Naples, Italy.
E-mail address: linda.sommese@unicampania.it (L. Sommese).

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HLA-G is a non-classic major histocompatibility complex class Ib antigen characterized by a low allelic polymorphism. Its expression is regulated through epigenetic mechanisms (alternative mRNA splicing), which produce different isoforms - four of them are membrane-bound (HLA-G1-G4), and three soluble (HLA-G5-G7); in addition, HLA-G1 isoform can produce a soluble form called sHLA-G1 derived from the membrane proteolytic shedding [9].

HLA-G expression was initially described in the maternal-fetal interface; it protects the fetus from destruction by its mother's immune system [10,11]. Afterwards, it was shown to contribute to tumor escape. It was also shown that HLA-G was involved in the protection of the transplanted tissues *via* the inhibition of all immune effectors that mediate graft rejection [12].

Detecting it may also serve as a clinical marker in predicting viral infections [13–16]. Hepatitis C virus (HCV) infection is a worldwide public health problem. Current dogma suggests that immunity to infection is controlled by T helper type (Th1 and Th2)-type immunity; Th1 and Th2 cell subsets are crucial in determining cytokine release [17,18]. In addition, several studies observed a high plasma soluble HLA-G (sHLA-G) levels in patients with chronic HCV infections; therefore, HLA-G protein could be considered a potential candidate involved in modulating susceptibility to HCV persistence and chronicity [16,19–21]. It was demonstrated that HLA-G acts on all immune response cells [22]; indeed, it was proved that HLA-G molecules inhibit cytotoxic activity of T cells, natural killer cell lysis, alloproliferative response, maturation of dendritic cells and can also be involved in generating regulatory cells [22].

Studies showed that high expression of sHLA-G on monocytes was associated with kidney allograft acceptance and that the presence of sHLA-G dimers linked to the lower levels of pro-inflammatory cytokines plays a potential role in controlling the inflammatory state [23,24]. HLA-G molecule has a high potential function to modulate the immune response towards the improvement of kidney graft survival in pre- and post-transplantation [22,25–28]. The up-regulation of sHLA-G expression in kidney transplant recipients without rejection compared to those with rejection was confirmed [29,30].

The aim of this study was to evaluate the expression of sHLA-G levels both in patients on the waiting list for kidney transplantation and in patients who returned in the list after the first transplantation by comparing them with blood donors as a control group. In addition, we investigated the correlation between sHLA-G levels and the presence of antibodies against HCV (anti-HCV).

2. Materials and methods

2.1. Study population

From June 2014 to May 2015, a total of 67 patients on the waiting list for kidney transplantation (53 men and 14 women) with a mean age of 56.2 ± 9.8 (range 26–77 years) with anti-HCV (n = 43) and without anti-HCV (n = 24) were included in this study. Among these patients, n = 39 were on the waiting list for the first transplantation (n = 25 with anti-HCV and n = 14 without anti-HCV), while n = 28 patients returned in the list after the first transplantation (n = 18 with anti-HCV and n = 10 without anti-HCV). The control group included n = 23 blood donors (17 men and 6 women) with mean age of 50.5 ± 9.3 (range from 32 to 65 years) with anti-HCV (n = 13) and without anti-HCV (n = 10). Serum samples from the subjects were preserved at −70 °C following centrifugation until assayed. A written consent was obtained from all patients. Both study groups and the control group did not show any HCV infection. In absence of major comorbidity contraindication renal transplantation, no description of clinic outcomes of patients and control group was reported.

2.2. Serum screening for HCV

Serum samples were tested for anti-HCV by chemiluminescent immunoassays (CMIA) on the ARCHITECT platform (Abbott Diagnostics, Wiesbaden, Germany) followed by HCV specific immunoblot assays as confirmatory testing (INNO-LIA, Innogenetics, Ghent, Belgium). Furthermore, all sera were also screened by NAT for HCV-RNA with the TaqScreen method on the Cobas s201 system (Roche Molecular Systems, Branchburg, NJ, USA): the assay was performed on mini pools of six samples each and has a nominal sensitivity of < 20 IU/mL. Each assay was performed in a single run for each specimen, and was carried out according to the respective manufacturer's instructions [31,32].

2.3. sHLA-G levels

Serum sHLA-G expression was determined with the sHLAG-specific ELISA kit (sHLA-G kit; BioVendor, Czech Republic) which measures HLA-G1 and HLA-G5. Each sample (100 µl) was measured in triplicate. The optical densities were measured at 450 nm (TECAN Infinite M200 station). Finally, sHLA-G concentrations (U/mL) in the samples were calculated using the calibration curve constructed by plotting the ODs against concentrations of calibrators provided by the manufacturer. The detection limit of ELISA kit was 0.6 U/mL. Details of the performance were according to the manufacturer's instruction.

2.4. Cytokine analysis

TNF-α and IL-10 serum levels were measured using enzyme-linked immunosorbent assay (ELISA) (pg/ml) (ELISA, R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations. Each sample (100 µl) was measured in triplicate. The minimum detectable dose (MDD) of TNF-α ranged from 0.5 to 5.5 pg/mL and the mean MDD was 1.6 pg/mL. The MDD of human IL-10 is typically less than 3.9 pg/mL. The values were read at 450 nm in an ELISA reader, and TNF-α and IL-10 concentrations were calculated from specific calibration curves prepared with known standard solutions.

2.5. Statistical analysis

All statistical analyses were carried out using SPSS 13.0. Box-plot of sHLA-G was organized by control group, patients on the waiting list for the first transplantation, and patients who returned in the list after the first transplantation. Data are expressed as median ± SD. Mean differences of sHLA-G between groups were estimated using independent sample *t*-test. Normal distribution of scalar parameters was assessed by Kolmogorov-Smirnov test. All parameters were normally distributed. Differences between anti-HCV negative and positive patients in sHLA-G were estimated using independent sample *t*-test. Pearson correlation was used to evaluate the correlation between sHLA-G and TNF-α, IL-10 and age in all patients with absence or presence of anti-HCV. A value of $p < 0.05$ was considered statistically significant.

2.6. Ethical approval

All procedures performed in this study were in accordance with the ethical standards of our institutional research and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients involved in this study.

Although our work was conducted under conformity of Declaration of Helsinki and no additional blood samples were taken, for all activities including the waiting list for organ transplantation and/or blood donation the approved Number of Ethic Committee, University of Campania "Luigi Vanvitelli" is Number: 295.