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Soluble HLA-G in Heart Transplantation: Their Relationship to Rejection Episodes and Immunosuppressive Therapy

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ABSTRACT: The aims of this study were to quantify the level of soluble HLA-G in heart transplant patients, to determine the relationship between the sHLA-G levels and the appearance of acute rejection episodes, and to identify the influence of immunosuppressive therapy on sHLA-G levels. Analysis of sHLA-G, measured by enzyme-linked immunosorbent assay in the transplant patients, revealed the existence of two similarly sized groups of patients. One group displayed a significant increase ($p < 0.001$) in sHLA-G during the first month after transplantation while the other group maintained low levels of the molecule (0–30 ng/ml) throughout the study. The latter group displayed a high incidence of recurrent severe rejection. A significant increase ($p < 0.01$) in sHLA-G 2 hours after administration of immunosuppressive treatment (mycophenolate mofetil, cyclosporine A/FK506, corticoids) was found. These results suggest that sHLA-G participates in the induction of certain levels of immunological tolerance in these recipients. *Human Immunology 67, 257–263 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: HLA; sHLA-G; heart transplantation; cyclosporine; tolerance; immunosuppressive treatment

ABBREVIATIONS

sHLA-G	soluble human leukocyte antigen-G
MMF	mycophenolate mofetil
APC	antigen-presenting cells
CsA	cyclosporine A
ELISA	enzyme-linked immunosorbent assay
MPA	mycophenolic acid
NK	natural killer
MP	methylprednisone

INTRODUCTION

One of the keys to successful organ transplantation is the prevention of their rejection as a consequence of graft allo-recognition of the donor allo-antigens by the recipient immune system [1]. Allo-recognition and the subsequent allo-response are complex processes that are currently minimized by means of immunosuppressive treatment [2]. The immunosuppressive treatment in current use comprises calcineurin inhibitors, steroids, antimetabolites, antiproliferatives, and other components such as monoclonal antibodies [3].

The common mechanism of action of the immunosuppressive treatment, which is based on nonspecific blockage of the immune system, displays serious limitations, including a high incidence of viral infections and the appearance of certain tumors [4]. In view of these limitations, efforts to specifically block the allo-recognition and the allo-response to the graft antigens are currently being made [5], while maintaining the functions of the immune system as a whole. This process, based on therapeutic tolerance, may protect the graft more efficiently, avoiding side effects. A large number of studies are currently attempting to make therapeutic improvements in this tolerogenic direction [6–8].

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TABLE 1 Clinical characteristics of the heart transplant patients of Group 2

Patients	Type of treatment	Cause of transplant	Age	Sex	Acute rejection*
T21	CsA, MMF,MP.	IM	49	M	+
T22	CsA, MMF,MP.	IDCM	38	M	+
T23	CsA, MMF,MP.	IDCM	24	M	−
T24	CsA, MMF,MP.	IM	40	M	−
T25	CsA, MMF,MP.	IDCM	51	M	−
T26	CsA, MMF,MP.	CM	28	M	−
T27	FK506, MMF,MP.	DCM	17	F	−
T28	CsA, MMF,MP.	IM	60	M	+
T29	CsA, MMF,MP.	DCM	52	M	+
T30	CsA, MMF,MP.	IDCM	49	M	+
T31	FK506, MMF,MP.	IDCM	16	M	−
T32	CsA, MMF,MP.	IM	37	F	−
T33	FK506, MMF,MP.	DCM	48	M	+
T35	CsA, MMF,MP.	IDCM	59	F	+
T36	CsA, MMF,MP.	DCM	41	M	−
T37	FK506, MMF,MP.	IM	39	F	−

IM, ischemic cardiomyopathy; IDCM, ideopathic dilated cardiomyopathy; DCM, dilated cardiomyopathy; CM, congenital cardiomyopathy; M/F, male/female.
* During the first month after transplantation.

HLA-G is a molecule with tolerogenic capacity in both its membrane and its soluble isoforms [9]. Specifically, the tolerogenic capability of soluble HLA-G (sHLA-G) is related to its capacity to induce apoptosis of activated CD8 T cells [10], to act on T regulatory cells [11], and to modulate the activity of natural killer cells [12]. HLA-G also has the ability to block allo-cytotoxic T lymphocyte response [13] and allo-proliferation [14], which suggests that this molecule could be involved in transplantation tolerance. In fact, high levels of sHLA-G in serum of transplant patients have been associated with better acceptance of combined liver–kidney transplants [15]. In heart transplant patients, although their levels of sHLA-G have not yet been quantified, it has been demonstrated that there is a relationship between the presence of this molecule in the serum of transplanted patients and a lower rate of rejection [16]. The aims of this study were to accurately measure soluble HLA-G in heart transplant patients by means of ELISA and to relate sHLA-G levels to episodes of acute rejection. We also investigated a possible link between soluble HLA-G expression and immunosuppressive therapy received by heart transplantation patients.

MATERIALS AND METHODS

Patients

This was a collaborative study of the Spanish Transplant Immunotolerance Group (RED-GIT). Serum samples were obtained from 20 healthy individuals (control group), 19 heart transplant patients from the Virgen de la Arraixaca Hospital in Murcia (Group 1), age range 25–63 years, and 16 heart transplant patients from the Reina Sofía University Hospital in Cordoba (Group 2), age range 16–60 years (Table 1). Heart transplantation had been performed as consequence of dilated cardiomyopathy in 15 patients, idiopathic dilated cardiomyopathy in 6 patients, ischemial cardiomyopathy in 11 patients, and congenital heart disease and other causes in 3 patients. No patient was found to have specific HLA antibodies before the transplant. The degree of rejection was classified according to the working formulation of the International Society for Heart and Lung Transplantation [17].

Immunosuppressive Therapy

Immunosuppressive treatment consisted of cyclosporine (CsA) or tacrolimus (FK506), methylprednisone (MP), and mycophenolate mofetil (MMF). *CsA* was started at a dosage of 4 mg/kg/day (administered as two doses) within the first 24 hours following transplantation, after which the dose was adjusted to maintain target C_0 levels: 1 month, 200–400 ng/ml; 2–3 months, 200–300 ng/ml; 4–6 months, 150–250 ng/ml; 6–12 months, 100–200 ng/ml. CsA levels were measured using fluorescence polarization immunoassay (Axsym). Additional immunosuppression consisted of corticosteroids: methylprednisolone 500 mg IV intra-operatively, 125 mg × three doses IV on day 1, followed by oral deflazacort, tapering to 0.2–0.3 mg/kg/day by 1 month, 0.15–0.20 mg/kg/day by 3 months, 0.1–0.2 mg/kg/day by 6 months, and then 0.05 mg/kg/day; and MMF at a starting dose of 1 g b.i.d, adjusted according to the mycophenolic acid (MPA) target range of 2.5–4.5 μg/ml. MPA levels were measured by homogeneous enzyme immunoassay. *Tacrolimus* was administered for the first 1–3 days after transplantation at a dose of 0.3 mg/kg/day in two di-