Human Immunology 71 (2010) 182–185

Contents lists available at ScienceDirect

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

Blood soluble human leukocyte antigen G levels are associated with human immunodeficiency virus type 1 infection in Beninese commercial sex workers

Julie Lajoie [a,b], Marguerite Massinga Loembe [c,d], Johanne Poudrier [a,b], Fernand Guédou [d], Jacques Pépin [e], Annie-Claude Labbé [b,f], Michel Alary [d], Michel Roger [a,b,*]

[a] Laboratoire d'Immunogénétique, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, Canada
[b] Dèpartement de Microbiologie et Immunologie de l'Université de Montréal, Montreal, Canada
[c] Laboratory of Immunology, Institute of Tropical Medicine, Antwerp, Belgium
[d] Unité de Recherche en Santé des Populations, Centre Hospitalier Affilié Universitaire de Québec and Université Laval, Quebec, Canada
[e] Dèpartement de Microbiologie et Infectiologie, Université de Sherbrooke, Sherbrooke, Canada
[f] Dèpartement de Microbiologie de l'Hôpital Maisonneuve-Rosemont, Montreal, Canada

ARTICLE INFO

Article history:
Received 15 September 2009
Accepted 5 November 2009
Available online 12 November 2009

Keywords:
African
HIV-1
HLA-G
Level of expression
Genetic variant
Immune regulator

ABSTRACT

Human leukocyte antigen (HLA)-G is a powerful modulator of the immune response. The aim of this study was to investigate whether soluble HLA-G (sHLA-G) expression is associated with human immunodeficiency virus type 1 (HIV-1) infection. HIV-1-infected female commercial sex workers (CSWs) had significantly lower levels of plasma sHLA-G compared with those in both the HIV-1-uninfected CSW and the non-CSW groups. The presence of HLA-G*010101, HLA-G*010404 alleles, and the 3′-untranslated region (3′UTR) genetic variant at position 3,952 were all significantly associated with lower plasma sHLA-G levels in the HIV-1-infected CSWs, whereas the HLA-G 3′UTR 14-bp sequence insertion was also associated with lower plasma sHLA-G levels in the overall population. When adjustment was made for all significant variables, the reduced expression of sHLA-G in the plasma remained significantly associated with HIV-1 infection and the HLA-G 3′UTR 14-bp insertion homozygote genotype. This study demonstrates that low levels of plasma sHLA-G are associated with HIV-1 infection.

1. Introduction

Human leukocyte antigen (HLA)-G is a nonclassical major histocompatibility class I protein, characterized by limited polymorphism and tissue-restricted distribution. HLA-G is expressed as membrane-bound (HLA-G1, -G2, -G3, and -G4) and soluble (HLA-G5, -G6, and -G7) isoforms because of alternative splicing [1]. The major isoforms present in the plasma are soluble HLA-G (sHLA-G)-1 and -G5, which are generated by shedding or proteolytic cleavage of the membrane-bound HLA-G1 isoform and by secretion of a soluble form, respectively. Under physiologic conditions, sHLA-G expression is associated with gender and HLA-G genetic variants. The level of sHLA-G is higher in women than in men [2]. Healthy individuals carrying the HLA-G*010103 and HLA-G*0105N alleles have lower plasma sHLA-G levels than subjects carrying the more frequently reported HLA-G*010101 allele. In addition, individuals with the latter allele have lower plasma sHLA-G levels than those with the HLA-G*010401 allele [3]. Polymorphisms in the 3′-untranslated region (3′UTR) can also affect the production of HLA-G molecules. The presence of a 14-bp sequence insertion in the HLA-G 3′UTR has been associated with lower levels of sHLA-G in the serum of healthy subjects [4,5]. HLA-G expression can be induced during pregnancy and under pathologic conditions such as autoimmune diseases, cancers, transplantations, and viral infections [6]. sHLA-G molecules inhibit natural killer and $CD8^+$ T-lymphocyte cytotoxic activity, as well as $CD4^+$ T-cell functions [7–9]. The immunosuppressive properties of HLA-G might contribute to the susceptibility of human immunodeficiency virus type 1 (HIV-1) infection. Recent studies have demonstrated that HLA-G polymorphisms are associated with an altered risk of heterosexual acquisition [10,11] and vertical transmission [12,13] of HIV-1. However, no data are available on the possible association between HLA-G expression at the protein level and susceptibility to HIV-1 infection. We have therefore measured the plasma levels of sHLA-G in HIV-1-infected and HIV-1-uninfected female commercial sex workers (CSWs) as well as HIV-1-uninfected non-CSW women at low risk for exposure to investigate whether sHLA-G expression is associated with HIV-1 infection.

2. Subjects and methods

Female CSWs were recruited consecutively from a dedicated sex worker clinic in Cotonou, Benin, and divided into two groups: HIV-1-uninfected CSWs ($n = 49$) and HIV-1-infected CSWs ($n = 20$). The HIV-1-uninfected non-CSW women ($n = 67$) were enrolled from a general health clinic in Cotonou. Written informed consent

* Corresponding author.
E-mail address: michel.roger.chum@ssss.gouv.qc.ca (M. Roger).

0198-8859/10/$32.00 - see front matter © 2010 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2009.11.007

Table 1
Distribution of demographic, sexual behavior, and genital tract infection characteristics in HIV-1-uninfected and HIV-1-infected CSWs and HIV-1-uninfected non-CSW control subjects

	HIV-1-uninfected CSWs $N = 49$	HIV-1-infected CSWs $N = 20$	HIV-1-uninfected non-CSW controls $N = 67$	p value[a]
Age, mean (SD), years	34.3 (12.3)	30.8 (8.3)	31.9 (9.3)	NS
Duration of sex work, mean (SD), years	4.3 (3.1)	4.2 (3.2)	NA	NS
Number of clients in the previous week, mean (SD)	16.9 (14.1)	10.1 (11.3)	NA	0.05
Days since last menses, mean (SD)	14.9 (8.3)	17.9 (17.7)	19.6 (12.7)	NS
Regular partner	29/47 (68%)	23/34 (68%)	54/67 (81%)	NS
Vaginal douching	46/49 (94%)	31/33 (94%)	61/67 (91%)	NS
Condom always used with clients	41/47 (87%)	24/30 (80%)	NA	NS
Vaginosis	19/22 (86%)	15/16 (94%)	20/45 (44%)	NS
Candidiasis	4/38 (11%)	4/22 (18%)	12/53 (23%)	NS
NG and/or CT infection	6/37 (16%)	5/25 (20%)	2/63 (3%)	NS

CSW = commercial sex worker; HIV-1 = human immunodeficiency virus type 1; N = number of participants; NA = nonapplicable; NS = nonsignificant; NG/CT = *Neisseria gonorrhoeae/Chlamydia trachomatis*.
All risk factor data were collected via a questionnaire administered before samples were collected. Gynecologic exams and biologic sampling were performed by a physician without knowledge of HIV status of the women to avoid potential bias.
[a] p values for the comparison across all groups were calculated with one-way analysis of variance for age, days since last menses; Mann–Whitney U test for the duration of sex work, and average number of clients; χ^2 test for regular partner, vaginal douching, condom use, vaginosis, candidiasis, and NG/CT.

was obtained from all subjects and the study was approved by the Ministère de la Santé du Bénin and by the CHUM human research ethics board. Women were excluded from the study if they were younger than 18 years old, menstruating, or pregnant. All study participants were antiretroviral therapy naive at the time of recruitment. Full methods for recruitment, baseline characteristics collection, and diagnostic laboratory procedures have been described elsewhere [14]. sHLA-G plasma levels were measured using a human sHLA-G immunoassay kit (Alexis Biochemicals, San Diego, CA), which allows simultaneous detection of HLA-G1 and -G5 soluble proteins without discrimination. DNA extraction was performed on peripheral blood mononuclear cells (PBMC) or dried blood spots using a Masterpure DNA extraction kit (Epicentre, Madison, WI). HLA-G polymorphism was determined by direct DNA sequencing analysis as described previously [11,12]. The positions of the polymorphisms reported in the current study are in reference to the wild-type HLA-G reference sequence (GenBank Accession No. J03027). Statistical analysis was performed using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, CA). Differences were considered significant at $p \leq 0.05$ or $p \leq 0.015$ when comparing two or three groups, respectively.

3. Results

Sociodemographic and clinical characteristics of the study population are described in Table 1. These data were collected to address the issue of confounding variables for risk of HIV-1 infection. The three groups were similar with respect to age, days from last menses, vaginal douching, and the presence of vaginosis, candidiasis, Chlamydia trachomatis, and Neisseria gonorrhoeae infection (Table 1). The average numbers of clients during the previous week was higher in HIV-1-unifected CSWs than in HIV-1-infected CSWs ($p = 0.05$), whereas the duration of sex work and condom use were equivalent between the two CSW groups.

Plasma sHLA-G levels were associated with HIV-1 infection. Indeed, HIV-1-infected CSWs had significantly lower levels of plasma sHLA-G (107 ± 102 U/ml) than did the HIV-1-uninfected CSWs (284 ± 213 U/ml; $p = 0.002$) and the HIV-1-uninfected non-CSW women (245 ± 166 U/ml; $p < 0.0001$; Fig. 1). There was no significant correlation between the HIV-1 viral load and the sHLA-G level in the plasma of HIV-1-infected CSWs as determined by Spearman's rank test ($r^2 = 0.048$, $p = 0.935$).

Because sHLA-G expression can also be associated with HLA-G polymorphism [3–5,15], we looked at the distribution of sHLA-G levels, either between study groups or in the total population, according to the HLA-G genetic variants (Table 2). HLA-G*010101 and G*010404 genotypes, in the heterozygous or homozygous states, were associated with reduced expression of sHLA-G in HIV-1-infected CSWs compared with individuals in both the HIV-1-uninfected CSW ($p = 0.018$ and $p = 0.0007$, respectively) and the non-CSW ($p = 0.006$ and $p = 0.015$, respectively) groups. Similarly, the homozygote genotype for the HLA-G 3′UTR variant at nucleotide position 3,952 was also associated with a reduced expression of sHLA-G in HIV-1-infected CSWs compared with those in both the HIV-1-uninfected CSW ($p = 0.0003$) and the non-CSW ($p = 0.0002$) groups. Although the 3′UTR 14-bp polymorphism was not associated with the sHLA-G expression patterns observed between the study groups, the homozygote genotype was associated with reduced expression of sHLA-G in the overall population ($p = 0.042$). Multiple logistic regression was used to define independent predictors of sHLA-G expression levels identified as significant in the crude analysis (HIV-1 infection, presence of HLA-G*010101, HLA-G*010404, HLA-G 3′UTR 14-bp sequence insertion, and variant at position 3,952). When adjustment was made for all significant variables, the reduced expression of sHLA-G in the plasma remained significantly associated with HIV-1 infection ($p = 0.012$) and the HLA-G 3′UTR 14-bp insertion homozygote genotype ($p = 0.03$).

Because HLA-G polymorphisms can be associated with HIV-1 infection [10–13], we also looked at the distribution of the HLA-G

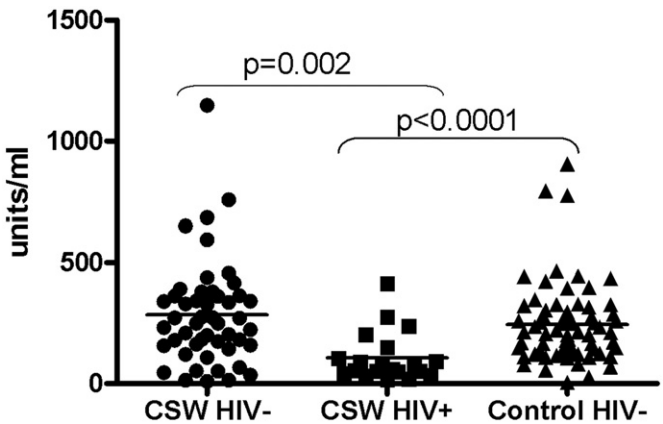

Fig. 1. Mean plasma soluble HLA-G levels according to the study groups. Statistical significance of differences in the plasma levels was evaluated with the Mann–Whitney U test. CSW, commercial sex worker; HIV-1, human immunodeficiency virus type 1.

Table 2
Plasma soluble HLA-G levels in HIV-1-uninfected and HIV-1-infected CSWs and HIV-1-uninfected non-CSW control subjects according to HLA-G gene polymorphism

HLA-G	HIV-1-uninfected CSWs		HIV-1-infected CSWs		HIV-1-uninfected non-CSWs		*p* value[a]	Total population		*p* value[b]
	N	Levels	*N*	Levels	*N*	Levels		*N*	Levels	
Allele[c]										
010101	29	317 (244)	11	118 (122)	33	232 (166)	0.009	73	249 (205)	NS
010102	15	336 (273)	5	155 (159)	12	232 (193)	NS	32	269 (234)	NS
0103	8	195 (141)	6	112 (87)	8	202 (130)	NS	24	175 (125)	NS
010401	5	328 (160)	2	141 (137)	15	320 (251)	NS	22	305 (224)	NS
010404	17	294 (190)	8	68 (47)	14	229 (123)	0.001	39	224 (168)	NS
0105N	8	265 (143)	1	83 (0)	8	168 (94)	NS	17	209 (127)	NS
3′UTR SNP										
3777 (c/c)	13	251 (172)	5	116 (117)	16	227 (195)	NS	34	243 (181)	NS
3952 (a/a)	30	287 (221)	11	76 (67)	32	236 (152)	0.0003	73	246 (204)	NS
14-bp (I/I)	5	206 (173)	3	103 (88)	5	165 (67)	NS	13	145 (98)	0.042

CSW = commercial sex worker; HIV-1 = human immunodeficiency virus type 1; I = insertion, *N* = number of participants; NS = nonsignificant; SNP = single nucleotide polymorphism; UTR = untranslated region. Data are means (SD).
[a] *p* values for the comparison between all groups were calculated with one-way analysis of variance test.
[b] *p* values were calculated with Mann–Whitney *U* test.
[c] Presence of the allele in the homozygous or heterozygous states.

genetic variants among the study groups and found no significant association between HLA-G polymorphism and HIV-1 infection (data not shown).

4. Discussion

We determined that a low level of plasma sHLA-G was associated with HIV-1 infection in Beninese CSW. This is in sharp contrast with the study of Donaghy *et al.*, which demonstrated elevated levels of sHLA-G in the blood of HIV-1-infected French subjects [16]. Most HIV-1-infected individuals analyzed in the latter study were on antiretroviral therapy, whereas all of our study participants were naive to treatment. Antiretroviral therapy can induce surface expression of HLA-G on blood peripheral monocytes from HIV-1-infected patients [17,18]. Hence, the relatively high blood levels of HLA-G observed in the French subjects could be caused by antiretroviral therapy and not HIV-1 infection per se. Moreover, the previous study [16] did not control for potential confounding factors such as gender [2], pregnancy [19], and HLA-G polymorphism [3–5,15] that could influence HLA-G expression. In the present study, all subjects were nonpregnant women and reduced expression of sHLA-G in the plasma was associated with certain HLA-G genotypes. However, most of the differences in sHLA-G expression attributable to the HLA-G genotype were observed between the HIV-1-infected and HIV-1-uninfected groups and the association between sHLA-G levels and HIV-1 infection remained significant when adjustment was made for the HLA-G genotype in multivariate analysis. HIV-1 can downregulate cell surface expression of the HLA-G1 molecule through a Nef-independent and Vpu-dependent mechanism [20,21]. sHLA-G molecules are generated by cleavage of the membrane-bound HLA-G1 isoform and by secretion of a soluble form (HLA-G5). Thus, it is possible that HIV-1 downregulation of membrane-bound HLA-G1 could reduce the number of available molecules for cleavage, consequently leading to the decreased plasma sHLA-G level observed in the HIV-1-infected CSW. However, the difference in HLA-G expression may partly be genetically determined. Indeed, the HLA-G 3′UTR 14-bp sequence insertion homozygote genotype was independently associated with a significantly reduced expression of plasma sHLAG in the overall population. The insertion of the 14-bp sequence in the 3′UTR of HLA-G has been associated with significantly reduced HLA-G mRNA levels [15] and lower levels of sHLA-G in the serum of healthy subjects [4,5].

In contrast to previous studies in Zimbabwean women [10,11], there was no significant difference in the distribution of HLA-G polymorphism among the HIV-1-infected and HIV-1-uninfected groups in the present study. The relatively small number of subjects analyzed in each group may have limited the power of the present study to reproduce our previous findings. Therefore, we cannot exclude the possibility that an HLA-G polymorphism such as the 3′UTR 14-bp sequence insertion could be associated with HIV infection by way of lowering sHLA-G.

Cytokine production could influence HLA-G expression and vice versa. Interleukin (IL)-10 induces HLA-G expression [22] and HLA-G can also stimulate IL-10 expression in peripheral blood monocytes [23]. Numerous studies have indicated that sHLA-G induces a shift toward a T_h1-type cytokine pattern. sHLA-G promotes the production of proinflammatory cytokines such as IL-6, IL-8, interferon-γ, and tumor necrosis factor (TNF)-α by decidual and peripheral blood natural killer cells [23–25]. Interestingly, we have previously measured the cytokine levels in the plasma samples of our study subjects and reported that HIV-1-infected CSWs had significantly lower levels of IL-2, IL-10, and TNF-α compared with those in both the HIV-1-uninfected CSW and the non-CSW groups. The same observation was made for IL-6 although the differences were not statistically significant [14].

Altogether, these results suggest that the expression of sHLA-G in the context of HIV-1 is a complex process modulated by many factors such as HIV-1, HLA-G genotypes, and cytokine expression patterns, which may contribute to an immunologic environment promoting the infection.

Acknowledgments

This work was supported by Grant HOP-79213 from the Canadian Institutes of Health Research (CIHR) and by the Réseau SIDA from the Fonds de la Recherche en Santé du Québec (FRSQ). Julie Lajoie and Marguerite Massinga Loembe hold Student Research awards from the CIHR. Marguerite Massinga Loembe additionally held a fellowship from the Agence Universitaire de la Francophonie. Michel Alary and Michel Roger are recipients of Research Scholar awards from the FRSQ. We are indebted to Nassirou Geraldo, Aina Gabin, Carmelle Assogba, and Clémence Agossa-Gbenafa for their clinical expertise, to Gérard Ahotin, Laurette Djossou, and Ella Goma for their technical assistance, and to Georges Batona and other field workers who helped with the recruitment of commercial sex workers.

References

[1] Ishitani A, Geraghty DE. Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. Proc Natl Acad Sci USA 1992;89:3947–51.
[2] Rudstein-Svetlicky N, Loewenthal R, Horejsi V, Gazit E. HLA-G levels in serum and plasma. Tissue Antigens 2007;69 (suppl 1):140–2.

[3] Rebmann V, van der Ven K, Passler M, Pfeiffer K, Krebs D, Grosse-Wilde H. Association of soluble HLA-G plasma levels with HLA-G alleles. Tissue Antigens 2001;57:15–21.
[4] Hviid TV, Hylenius S, Lindhard A, Christiansen OB. Association between human leukocyte antigen-G genotype and success of in vitro fertilization and pregnancy outcome. Tissue Antigens 2004;64:66–9.
[5] Hviid TV, Rizzo R, Christiansen OB, Melchiorri L, Lindhard A, Baricordi OR. HLA-G and IL-10 in serum in relation to HLA-G genotype and polymorphisms. Immunogenetics 2004;56:135–41.
[6] Pistoia V, Morandi F, Wang X, Ferrone S. Soluble HLA-G: are they clinically relevant? Semin Cancer Biol 2007;17:469–79.
[7] Le Gal FA, Riteau B, Sedlik C, Khalil-Daher I, Menier C, Dausset J, et al. HLA-G-mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int Immunol 1999;11:1351–6.
[8] Park GM, Lee S, Park B, Kim E, Shin J, Cho K, Ahn K. Soluble HLA-G generated by proteolytic shedding inhibits NK-mediated cell lysis. Biochem Biophys Res Commun 2004;313:606–11.
[9] Lila N, Rouas-Freiss N, Dausset J, Carpentier A, Carosella ED. Soluble HLA-G protein secreted by allo-specific $CD4^+$ T cells suppresses the allo-proliferative response: a $CD4^+$ T cell regulatory mechanism. Proc Natl Acad Sci USA 2001; 98:12150–5.
[10] Lajoie J, Hargrove J, Zijenah LS, Humphrey JH, Ward BJ, Roger M. Genetic variants in nonclassical major histocompatibility complex class I human leukocyte antigen (HLA)-E and HLA-G molecules are associated with susceptibility to heterosexual acquisition of HIV-1. J Infect Dis 2006;193:298–301.
[11] Matte C, Lajoie J, Lacaille J, Zijenah LS, Ward BJ, Roger M. Functionally active HLA-G polymorphisms are associated with the risk of heterosexual HIV-1 infection in African women. AIDS 2004;18:427–31.
[12] Aikhionbare FO, Kumaresan K, Shamsa F, Bond VC. HLA-G DNA sequence variants and risk of perinatal HIV-1 transmission. AIDS Res Ther 2006;3:28.
[13] Fabris A, Catamo E, Segat L, Morgutti M, Arraes LC, de Lima-Filho JL, Crovella S. Association between HLA-G 3′UTR 14-bp polymorphism and HIV vertical transmission in Brazilian children. AIDS 2009;23:177–82.
[14] Lajoie J, Poudrier J, Massinga-Loembe M, Guédou F, Agossa-Gbenafa C, Labbé AC, et al. Differences in immunoregulatory cytokine expression patterns in the systemic and genital tract compartments of HIV-1-infected commercial sex workers in Benin. Mucosal Immunol 2008;1:309–16.
[15] Hviid TV, Hylenius S, Rorbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 2003;55:63–79.
[16] Donaghy L, Gros F, Amiot L, Mary C, Maillard A, Guiguen C, Gangneux JP. Elevated levels of soluble non-classical major histocompatibility class I molecule human leucocyte antigen (HLA)-G in the blood of HIV-infected patients with or without visceral leishmaniasis. Clin Exp Immunol 2007;147:236–40.
[17] Cabello A, Rivero A, Garcia MJ, Lozano JM, Torre-Cisneros J, González R, et al. HAART induces the expression of HLA-G on peripheral monocytes in HIV-1 infected individuals. Hum Immunol 2003;64:1045–9.
[18] Rivero A, Lozano JM, Gonzalez R, García-Jurado G, Camacho A, Torres-Cisneros J, Peña J. Nucleoside reverse transcriptase inhibitors are able and protease inhibitors unable to induce the tolerogenic molecule HLA-G1 on monocytes from HIV-1 infected patients. Hum Immunol 2007;68:303–6.
[19] Hackmon R, Hallak M, Krup M, Weitzman D, Sheiner E, Kaplan B, Weinstein Y. HLA-G antigen and parturition: maternal serum, fetal serum and amniotic fluid levels during pregnancy. Fetal Diagn Ther 2004;19:404–9.
[20] Derrien M, Pizzato N, Dolcini G, Menu E, Chaouat G, Lenfant F, et al. Human immunodeficiency virus 1 downregulates cell surface expression of the non-classical major histocompatibility class I molecule HLA-G1. J Gen Virol 2004;85:1945–54.
[21] Pizzato N, Derrien M, Lenfant F. The short cytoplasmic tail of HLA-G determines its resistance to HIV-1 Nef-mediated cell surface downregulation. Hum Immunol 2004;65:1389–96.
[22] Moreau P, Adrian-Cabestre F, Menier C, Guiard V, Gourand L, Dausset J, et al. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol 1999;11:803–11.
[23] van der Meer A, Lukassen HG, van Cranenbroek B, Weiss EH, Braat DD, van Lierop MJ, Joosten I. Soluble HLA-G promotes Th1-type cytokine production by cytokine-activated uterine and peripheral natural killer cells. Mol Hum Reprod 2007;13:123–33.
[24] Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der Meer A, Joosten I, Long EO. Activation of NK cells by an endocytosed receptor for soluble HLA-G. PLoS Biol 2006;4:e9.
[25] Li C, Houser BL, Nicotra ML, Strominger JL. HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. Proc Natl Acad Sci USA 2009;106:5767–72.