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Expression of membrane-bound human leucocyte antigen-G in systemic sclerosis and systemic lupus erythematosus

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ABSTRACT

Human leucocyte antigen-G (HLA-G) is a nonclassical class I major histocompatibility complex (MHC) molecule characterized by complex immunoregulatory and tolerogenic functions. Membrane-bound HLA-G is expressed on the surface of different cell populations in both physiological and pathological conditions. Systemic sclerosis (SSc) is a multisystem autoimmune disease characterized by widespread tissue fibrosis, vascular lesions and immunological alterations. Systemic lupus erythematosus is the prototypic systemic autoimmune disease affecting virtually any organ system, such as skin, joints, central nervous system, or kidneys. In SSc and SLE patients, the membrane expression of HLA-G on monocytes (0.88 ± 1.54 and 0.43 ± 0.75, respectively), CD4+ (0.42 ± 0.78 and 0.63 ± 0.48, respectively), CD8+ (2.65 ± 3.47 and 1.29 ± 1.34, respectively) and CD4+ CD8+ double-positive cells (13.87 ± 15.97 and 3.79 ± 3.11, respectively) was significantly higher than in healthy controls (0.12 ± 0.07; 0.01 ± 0.01; 0.14 ± 0.20 and 0.32 ± 0.38, respectively) ($p < 0.0001$). Our results show that in SSc and SLE the membrane expression of HLA-G by different subpopulations of peripheral blood mononuclear cells (PBMC) is increased, suggesting a potential role of HLA-G molecules in the complex immunological pathogenesis of these two autoimmune disorders.

1. Introduction

Human leucocyte antigen (HLA)-G is a human nonclassical major histocompatibility complex (MHC) molecule expressed mainly in membrane-bound form at the foetal–maternal interface [1,2]. It is also expressed in a few adult tissues [3–6], as well as in different cells such as activated monocytes and erythroid and endothelial precursors [7]. The expression of HLA-G antigens has been also reported in some solid tumours, transplanted organs and cutaneous inflammatory diseases as well as on virally infected cells [8,9].

HLA-G seems to exert several immunoregulatory functions [10,11] and is currently considered as an 'immune checkpoint' molecule [12]. Of interest, HLA-G-positive regulatory T cells (Treg) have been detected in peripheral blood and inflamed tissues [13–16].

Systemic sclerosis (SSc) is a connective tissue disease characterized by diffuse fibrosis and vascular lesions occurring in skin and internal organs [17,18]. Although the causative factors remain to be characterized, three pathogenetic events underlie SSc development, namely vascular damage, immune dysregulation and fibroblast activation [19–21]. As far as the immune system is concerned, autoantibodies [22], high levels of B cell activating factor (BAFF) [23] and alterations of dendritic [24], T helper type 17 (Th17) and Treg subpopulations have been reported [25–27]. HLA-G molecules have been detected in approximately 50% of skin biopsies from SSc patients, and their expression has been associated with a better clinical outcome [28].

Systemic lupus erythematosus (SLE) is an autoimmune disease, that may affect skin, joints, kidneys, brain, and blood vessels [29]. Different immunological alterations have been described in patients affected by SLE, including polyclonal B lymphocyte activation, autoantibody production and defective T cell function [30–34].

Several HLA-G polymorphisms have been reported to be associated with susceptibility to the development of some immune-mediated diseases and their different clinical manifestations [35–39].

Our group and other researchers have already explored HLA-G expression in immune cells from SSc and SLE patients [40–43]. However, available data are scanty and sometimes conflicting. The aim of the

Abbreviations: ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; CD, cluster of differentiation; DP, double positive; HD, healthy donors; HLA, human leucocyte antigen; mAb, monoclonal antibody; SLE, systemic lupus erythematosus SSc, systemic sclerosis

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present study was to evaluate the expression of HLA-G in peripheral blood immune cells subpopulations in a cohort of SSc and SLE patients and compare their expression in these two diseases.

2. Materials and methods

2.1. Patients and controls

Forty-eight SSc patients (38 females and 10 males, aged 38–89 years) and twenty-four patients affected by SLE (all females, aged 27–69) followed at the Clinical Immunology Unit (Department of Internal Medicine, University of Genoa, Genoa, Italy) were included in the study. All SSc patients met the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) 2013 classification criteria for SSc [44]. In addition, SSc patients were categorized as having limited (lSSc, 34 subjects) or diffuse (dSSc, 14 subjects), according to the LeRoy criteria [45]. All SLE patients satisfied the 1997 updated ACR criteria for the classification of SLE [46]. Forty-three healthy donors (HD), matched for sex and age, were recruited as controls. This study was approved by the local Ethical Committee and all patients signed a written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2. Phenotypic analyses by flow cytometry

The expression of cell membrane antigens by peripheral blood mononuclear cells (PBMC) was analysed by direct immunofluorescence incubating 100 µl of peripheral blood from each individual with the fluorochrome-conjugated anti-HLA-G MEM-G9 monoclonal antibody (mAb) (Exbio, Vestec, Czech Republic), which reacts with the native form of HLA-G1, and with the fluorochrome-conjugated anti-CD3, -CD4, -CD8, -CD14 and -CD45 mAbs (Beckman Coulter Europe, Cassina de'Pecchi, Italy) at 4 °C for 30 min in the dark. Fluorochrome-conjugated isotype matched antibodies were used as controls. After red blood cell lysis, analysis was performed by flow cytometry using a Navios flow cytometer equipped with KALUZA software (Beckman Coulter Europe).

2.3. Statistical analysis

Values are expressed as median and mean ± standard deviation. Comparisons among HLA-G membrane expression in PBMC from SSc patients, SLE patients and healthy controls were performed by Mann-Whitney *U* test. P-values were considered significant when equal to or < 0.01. Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Evaluation of membrane-bound HLA-G expression in PBMC of SSc patients

The mean percentage of monocytes (CD45+ CD14+) did not differ significantly between SSc patients and healthy donors (Table 1). The percentage of HLA-G-expressing monocytes was significantly higher in SSc patients than in controls (Table 1 and Fig. 1, panel A). The percentage of CD4+ and CD8+ lymphocytes was similar in PBMC from SSc patients and controls (Table 1). The percentage of HLA-G positive CD4+ and CD8+ cells was significantly higher in PBMC from SSc patients compared to controls (Table 1 and Fig. 1, panels B and C). SSc patients showed a higher, but not significant, percentage of CD4+ CD8+ double-positive (DP) cells with respect to controls (Table 1). Inside the DP lymphocytes population, SSc patients displayed a significantly higher proportion of HLA-G-expressing cells compared to controls (Table 1 and Fig. 1, panel D). Furthermore, in the context of DP cells, the subpopulation of $CD4^{dull}CD8^{high}$ cells from SSc patients presented a high percentage of HLA-G+ cells (32.22 ± 24.78), whereas HLA-G membrane expression was undetectable in the same subpopulation of healthy controls. SSc samples were further analysed separating limited and diffuse forms of disease, but no significant differences were detectable in the percentage of HLA-G-positive cells among monocytes, CD4+, CD8+, DP and $CD4^{dull}CD8^{high}$ subpopulations (data not shown). Representative examples of HLA-G expression by monocytes, CD4+, CD8+, DP and $CD4^{dull}CD8^{high}$ cells from a SSc patient and a healthy donor are shown in Figs. 2 and 3.

3.2. Evaluation of membrane-bound HLA-G expression in PBMC of SLE patients

The percentage of monocytes (CD45+ CD14+) was similar in SLE patients and healthy donors (Table 1). The percentage of HLA-G-positive monocytes was significantly higher in SLE patients than in controls (Table 1 and Fig. 1, panel A). As expected in lupus disease, the proportion of CD4+ cells was lower than in healthy donors (Table 1) whereas the percentage of CD8+ was proportionally higher in SLE patients as compared to healthy subjects (Table 1). The percentage of HLA-G-expressing CD4+ and CD8+ lymphocytes was significantly higher in SLE patients with respect to healthy donors (Table 1 and Fig. 1, panels B and C). SLE patients had a percentage of DP cells similar to controls (Table 1). In the context of DP lymphocytes, SLE patients showed a significantly higher percentage of HLA-G positive cells than controls (Table 1 and Fig. 1, panel D). Moreover, inside the population of DP cells, $CD4^{dull}CD8^{high}$ cells from SLE patients displayed a high proportion of HLA-G+ cells (10.21 ± 13.02) although HLA-G was virtually absent in $CD4^{dull}CD8^{high}$ cells of healthy subjects. Figs. 2 and 3 exemplify the expression of membrane HLA-G by monocytes, CD4+, CD8+, DP and $CD4^{dull}CD8^{high}$ cells from a representative SLE patient and a healthy control subject.

3.3. Comparison of membrane-bound HLA-G expression in PBMC of SSc and SLE patients

The percentage of HLA-G-expressing monocytes was higher in SSc patients than in SLE patients (Table 1 and Fig. 1, panel A). The percentage of HLA-G+ CD4+ cells was lower in SSc as compared to SLE patients (Table 1 and Fig. 1, panel B). The percentage of HLA-G-expressing CD8+ cells was similar in SSc and SLE patients (Table 1 and Fig. 1, panel C). Both SSc and SLE patients had a similar percentage of DP cells (Table 1), but SSc patients displayed a significantly higher percentage of HLA-G+ DP cells than SLE patients (Table 1 and Fig. 1, panel D). Similarly, the proportion of HLA-G+ cells in the context of $CD4^{dull}CD8^{high}$ population was significantly higher in SSc patients with respect to patients affected by SLE (32.22 ± 24.78 *vs* 10.21 ± 13.02, respectively; p-value < 0.0001).

4. Discussion

In the present paper we have demonstrated that the percentage of HLA-G positive monocytes is increased in both SSc and SLE patients. This finding is in agreement with previous data reporting that mononuclear phagocytes from healthy subjects express HLA-G mRNA and protein and that interferon (IFN)-γ enhances HLA-G expression [47]. By contrast, they are in disagreement with other Authors who report a diminished expression of HLA-G and a defective response to IFN-γ stimulation by monocytes from SLE patients [40]. Moreover, we found that the percentage of CD4+ and CD8+ cells expressing HLA-G molecules was significantly higher in SSc and SLE patients than in controls. These HLA-G expressing cells are different from the naturally occurring CD25+ Forkhead box protein 3 (FoxP3+) Tregs and may be involved in the modulation of immune responses in inflammed tissues of patients affected by immune mediated disorders [14,48,49]. In addition, we detected a significant increase in HLA-G expression in DP cells in

Table 1
Median and mean ± standard deviation (SD) of total and HLA-G positive monocytes, CD4+ lymphocytes, CD8+ lymphocytes and CD4+ CD8+ lymphocytes from healthy donors (HD), scleroderma patients (SSc) and Systemic Lupus Erythematosus (SLE) patients.

	SSc (48) median mean ± SD	SLE (24) median mean ± SD	HD (43) median mean ± SD	SSc *vs* HD*	SLE *vs* HD*	SSc *vs* SLE*
Monocytes	7.60 7.41 ± 1.33	7.03 7.04 ± 2.75	7.36 7.31 ± 1.13	*ns*	*ns*	*ns*
Monocytes HLA-G+	0.69 0.88 ± 1.54	0.25 0.43 ± 0.75	0.09 0.12 ± 0.07	$p < 0.0001$	$p = 0.0008$	$p = 0.0002$
CD4+	71.0 72.45 ± 9.71	50.78 51.73 ± 16.21	72.0 70.63 ± 8.66	*ns*	$p < 0.0001$	$p < 0.0001$
CD4+ HLA+ G+	0.24 0.42 ± 0.78	0.46 0.63 ± 0.48	0.01 0.01 ± 0.01	$p < 0.0001$	$p < 0.0001$	$p = 0.0027$
CD8+	27.00 26.91 ± 9.21	37.14 39.91 ± 14.33	27.00 25.12 ± 6.32	*ns*	$p = 0.0011$	$p = 0.0001$
CD8+ HLA-G+	0.51 2.65 ± 3.47	0.74 1.29 ± 1.34	0.04 0.14 ± 0.20	$p < 0.0001$	$p < 0.0001$	*ns*
CD4+ CD8+	2.39 5.01 ± 7.12	1.48 2.79 ± 3.78	2.33 2.56 ± 1.87	*ns*	*ns*	*ns*
CD8+ CD4+ HLA-G+	14.09 13.87 ± 15.97	3.56 3.79 ± 3.11	0.35 0.32 ± 0.38	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

*Comparisons between HD, SSc and SLE patients were performed by Mann-Whitney *U* test.

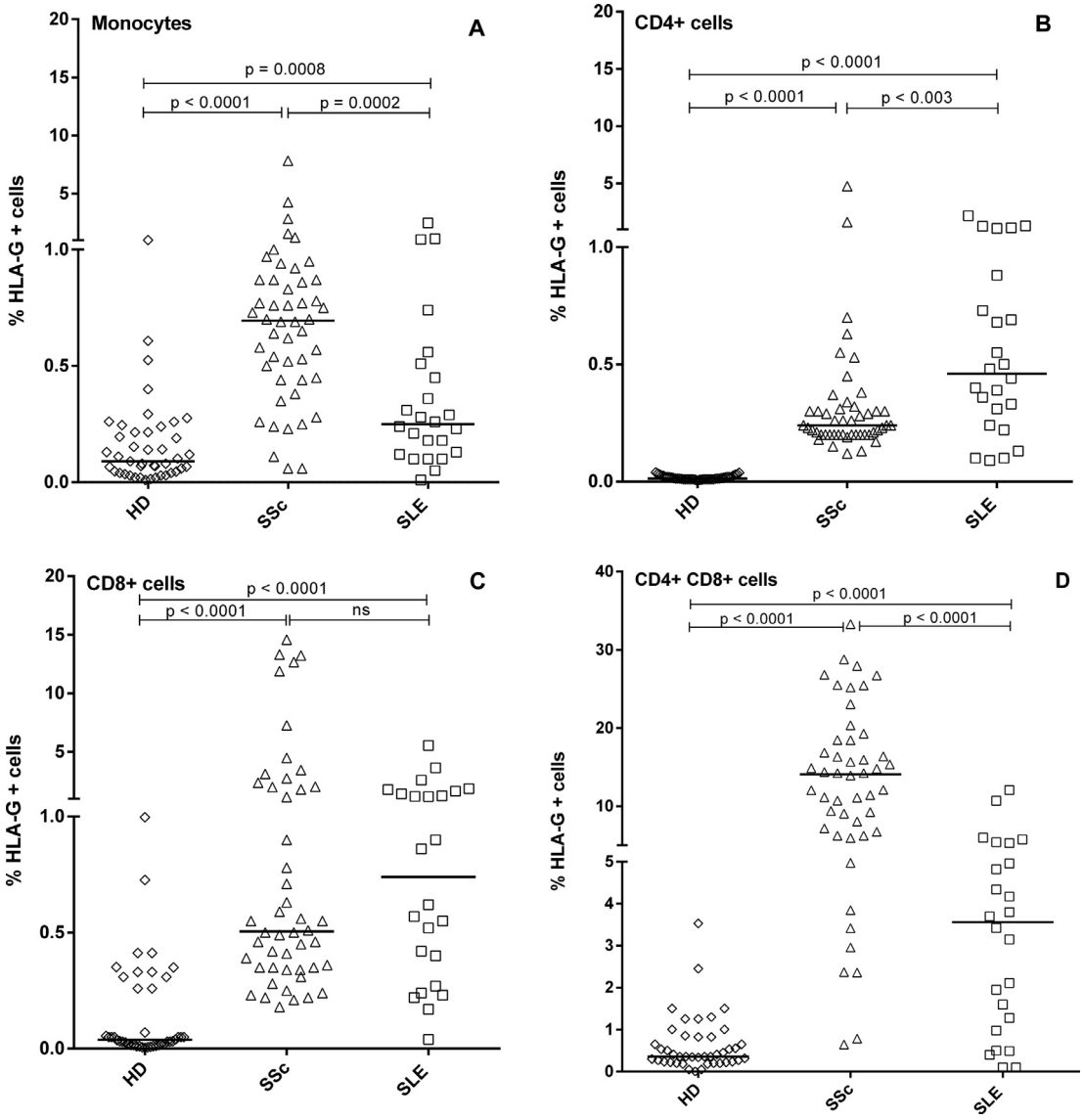

Fig. 1. Percentage of HLA-G positive cells in monocytes (panel A), CD4+ lymphocytes (panel B), CD8+ lymphocytes (panel C) and CD4+ CD8+ lymphocytes (panel D) from healthy donors (HD), systemic sclerosis patients (SSc) and systemic lupus erythematosus (SLE) patients. Horizontal bars represent median values.

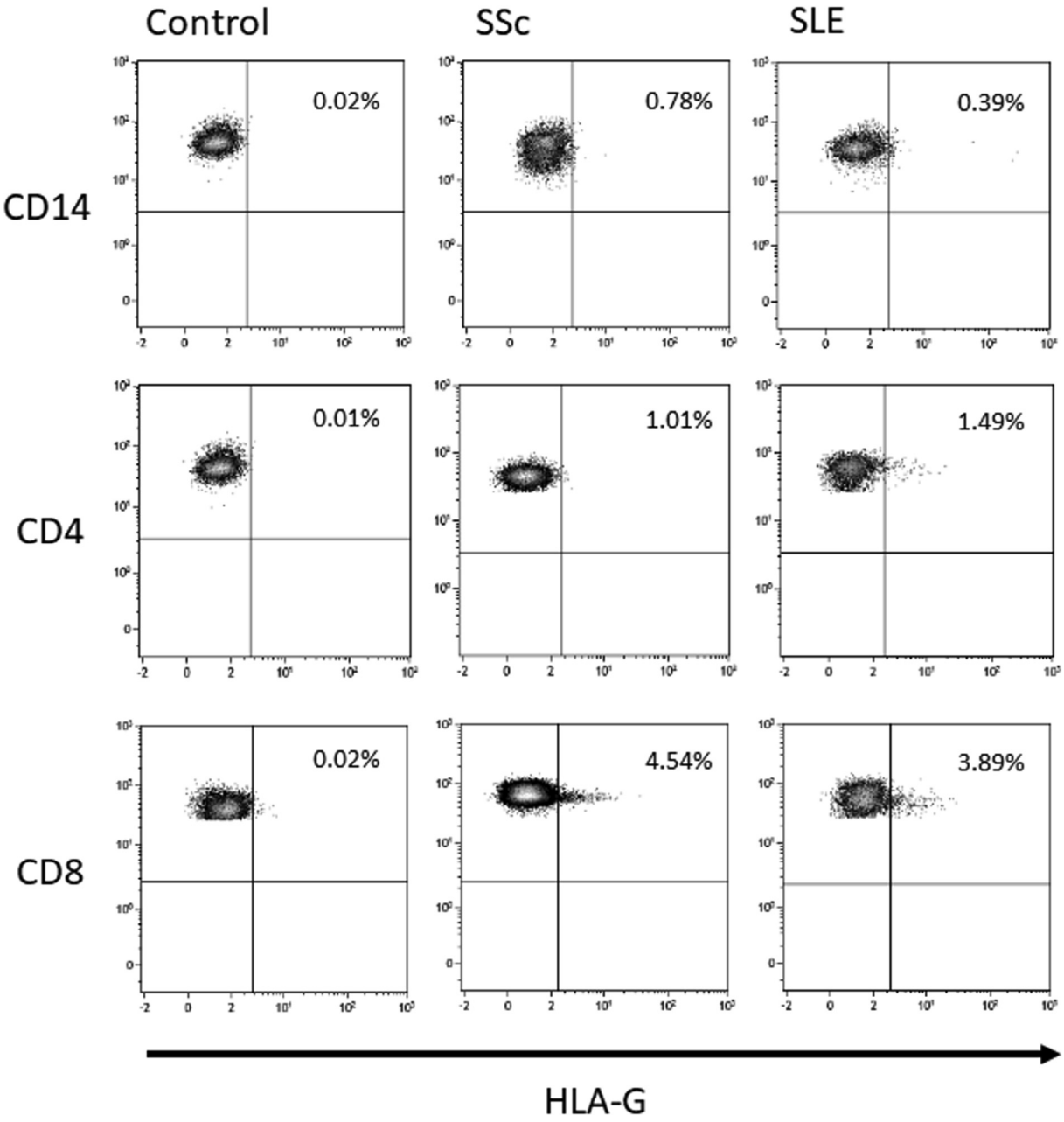

Fig. 2. Membrane expression of human leucocyte antigen-G (HLA-G) molecule on peripheral blood mononuclear cells from systemic sclerosis (SSc) and systemic lupus erythematosus patients and healthy donors.

patients affected by SSc and SLE compared to healthy subjects. The function of circulating DP cells remains controversial [50], however it is worthy of note that DP cells are present in the skin of patients with early active SSc and may contribute to the enhanced extracellular matrix deposition by fibroblasts [51] as well as in SLE where they exert a suppressive role in the production of autoantibodies [52]. Finally, a subpopulation of $CD4^{dull}CD8^{high}$ cells expressing HLA-G was detected among DP cells in SSc and SLE patients but was virtually absent in healthy controls. Of interest, the proportion of HLA-G positive cells was higher in SSc patients as compared with SLE patients. Clearly, we cannot exclude that HLA-G expression could be modulated by various treatment employed in our cohort of SSc and SLE patients. However, it is worth of note that SSc patients, the cohort in which HLA-G is expressed at the highest level, typically are not managed with immunosuppressive drugs or steroids.

5. Conclusions

Collectively, these data indicate that in SSc and SLE the membrane expression of HLA-G is significantly elevated in peripheral blood immune cells subpopulations. A possible involvement of HLA-G in SSc and SLE pathogenesis might be suggested by the immunosuppressive role of HLA-G molecules [53–56]. It may be proposed that the up-regulation of HLA-G membrane expression by PBMC could reflect an attempt to control the immune derangement occurring in systemic autoimmune diseases. This hypothesis is supported by data indicating that expression of HLA-G has been detected in the skin of SSc and SLE patients [28,41]. However, available data are inconclusive and further perspective basic and clinical investigations are required in order to define the role, if any, of HLA-G molecules in SSc and SLE pathogenesis and clinical course.

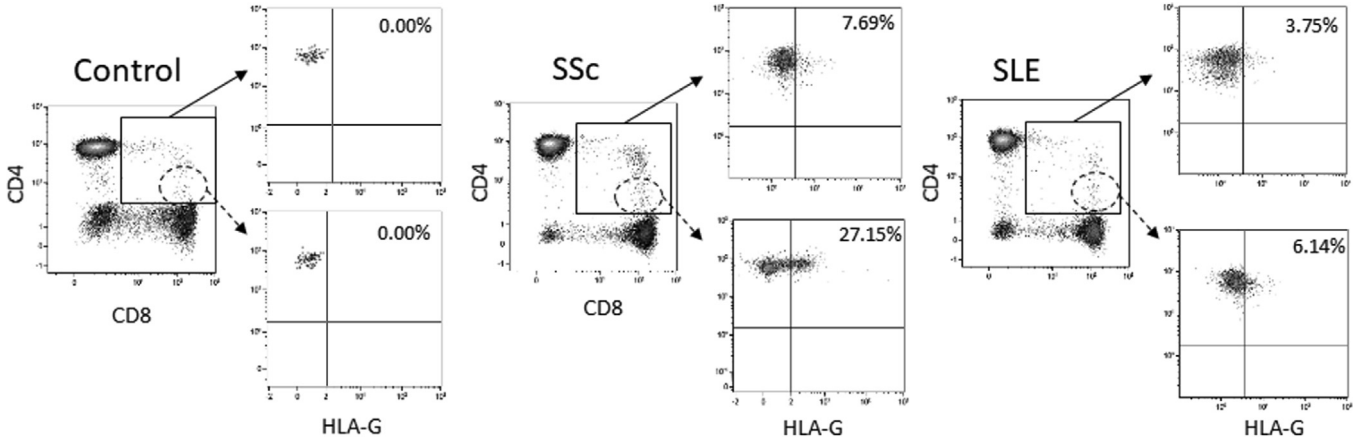

Fig. 3. Membrane expression of human leucocyte antigen-G (HLA-G) molecule on CD4+ CD8+ double positive (DP) and $CD4^{dull}CD8^{high}$ subpopulations from systemic sclerosis (SSc) and systemic lupus erythematosus representative patients and a healthy subject.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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