Human Immunology 80 (2019) 948–954

Contents lists available at ScienceDirect

Human Immunology

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

Human Antigen Leucocyte (HLA)-G and HLA-E are differentially expressed in pancreatic disorders

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ARTICLE INFO

Keywords:
Diabetes *Mellitus*
Chronic pancreatitis
Major histocompatibility complex
Immune checkpoint molecules

ABSTRACT

Background: Little information is available regarding the expression of the immunomodulatory Human Leukocyte Antigen (HLA)-G and -E molecules in pancreatic disorders.

Aim: To analyze HLA-G and -E expression in specimens of alcoholic chronic pancreatitis (ACP), idiopathic chronic pancreatitis (ICP), type 1 (T1D) and type 2 diabetes (T2D) and in histologically normal pancreas (HNP).

Methods: HLA-G and -E expression (ACP = 30, ICP = 10, T1D = 10, T2D = 30 and HNP = 20) was evaluated by immunohistochemistry in three different areas (acini, islets and inflammatory infiltrate).

Results: Acini and islets from HNP specimens exhibited higher HLA-G and -E expression compared to corresponding areas from all other patient groups. In inflammatory infiltrate, HLA-G and -E expression was observed only among the pancreatic disorders. We observed higher HLA-G and -E expression in acini from T2D compared to ACP, as well as higher HLA-G expression compared to ICP.

Conclusion: The decreased expression of HLA-G and -E in islets and acini together with the expression of these molecules in the inflammatory infiltrating cells were shared features among chronic inflammatory and autoimmune pancreatic disorders evaluated in this study, possibly reflecting tissue damage.

1. Introduction

The exocrine and endocrine components of the pancreas may be affected by autoimmune and chronic inflammatory disorders. Type 1 diabetes (T1D) arises from the autoimmune destruction of β-islet cells in genetically susceptible subjects [1], preventing peripheral tissues to grasp blood glucose [2]. Although type 2 diabetes (T2D) is caused by genetic and environmental factors that promote β-islet cell exhaustion, affecting the ability of peripheral tissues to respond to insulin, usually no significant morphological changes are observed in the pancreas [3]. Pancreatitis is an inflammatory condition in which pancreatic enzymes accumulate and destroy pancreatic cells, and chronic pancreatitis (CP) is characterized by progressive inflammation and fibrosis, resulting in loss of exocrine function with secondary damage to the pancreatic islets [4]. Although chronic alcoholism is considered to be responsible for the vast majority of CP, cases of idiopathic pancreatitis are also described [5].

Considering that the immune system cell infiltrate usually permeates the pathogenic features of autoimmune and chronic inflammatory pancreatic disorders [5–7], and considering that pancreas is one of the few organs that express the immune checkpoint Human Antigen Leucocyte (HLA)-G [8], the study of molecules that control the immune response is relevant. HLA-G and HLA-E are non-classical class I molecules that can be expressed both as membrane-bound and soluble isoforms [9]. Besides pancreas, HLA-G is also physiologically expressed in trophoblasts at the fetal-maternal interface [10–13], in the thymus and

Abbreviations: HLA, Human Antigen Leucocyte; CP, Chronic pancreatitis; ACP, Alcoholic chronic pancreatitis; ICP, Idiopathic chronic pancreatitis; T1D, Type 1 diabetes; T2D, Type 2 diabetes; HNP, Histologically normal pancreas; IHC, Immunohistochemistry; NK, Natural killer; T_{reg}, $CD4^+$ regulatory T cells; T_H17, $CD4^+$ T-helper 17 cells; T_H1, $CD4^+$ T-helper 1 cells; H&E, Hematoxylin and eosin; PBS, Phosphate-buffered saline; mAbs, Monoclonal antibodies; 5′URR, 5′ upstream region; LCR, Locus control region; NF-κB, Nuclear factor κB; IFN, Interferon; LINE1, Long interspersed elements

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https://doi.org/10.1016/j.humimm.2019.09.002
Received 2 April 2019; Received in revised form 28 August 2019; Accepted 3 September 2019
Available online 25 September 2019

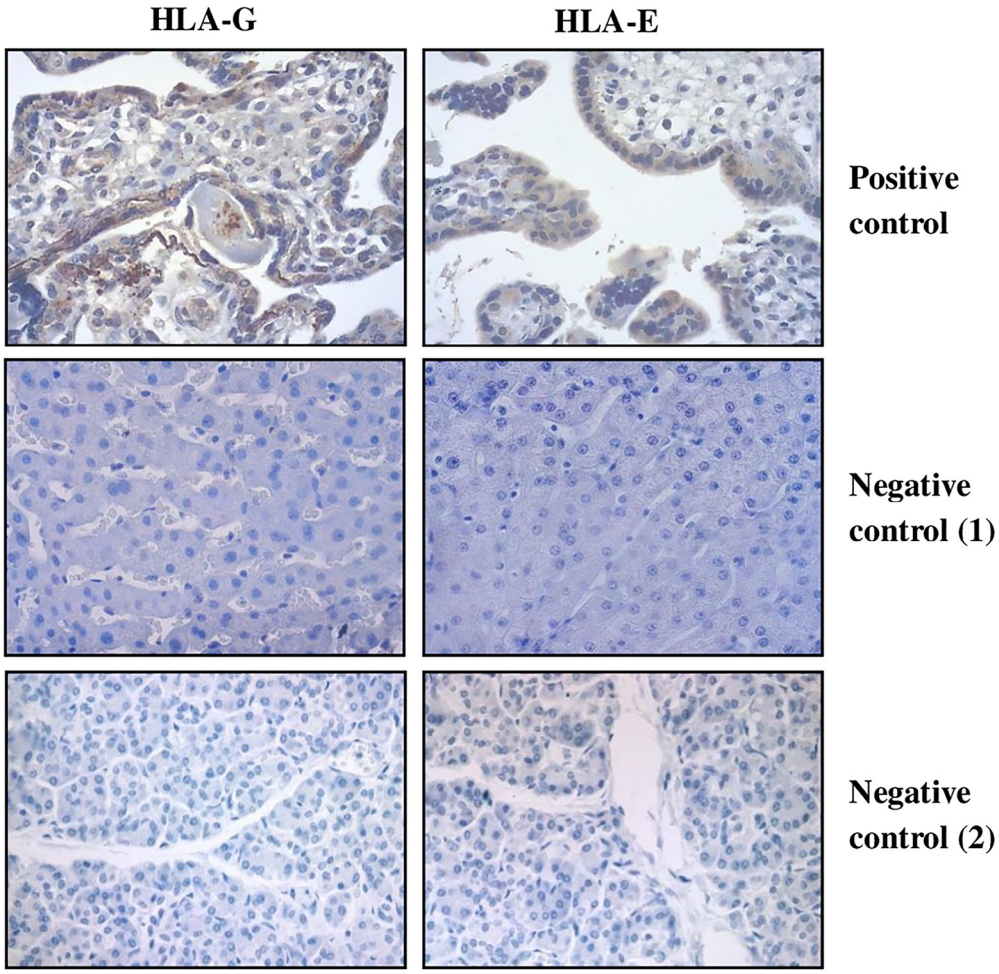

Fig. 1. Immunohistochemical staining for Human Antigen Leucocyte (HLA)-G and HLA-E. Positive control: Cytotrophoblast from first-trimester human placenta expressing both HLA-G and HLA-E (brown staining). Negative control (1): Absence of HLA-G and -E expression in rat liver tissue. Negative control (2): Histologically normal pancreas (HNP) performed by omitting the primary antibody for HLA-G and HLA-E. Original magnification ×200.

in few other tissues [14].

HLA-G is the best characterized non-classical class I molecule, which interacts with leukocyte receptors such as ILT2, ILT4 and KIR2DL4, usually inhibiting the function of several cells of the innate and adaptive immune system [15], including: i) the cytotoxic activity of $CD8^+$ T lymphocytes and natural killer (NK) cells, ii) the B lymphocyte proliferation and immunoglobulin production [16,17] and iii) the function or maturation of myeloid cells (monocytes/macrophages/dendritic cells) [18]. In addition, HLA-G may generate $CD4^+$ regulatory T cells (T_{reg}) [16,17].

HLA-E is defined by its low expression in healthy tissues that also express other HLA class I molecules (*i.e.,* HLA-A, -B, -C and -G) [9,19]. Indeed, HLA-E main function is to present peptides derived from the leader sequence of HLA class I molecules to NK cells and $CD8^+$ T lymphocytes through the interaction with CD94/NKG2A inhibitor receptor or CD94/NKG2C activator receptor, allowing these cytotoxic cells to monitor the level of HLA class I expression, inhibiting or not its effector functions [20].

Considering that the reason by which HLA-G is constitutively expressed in pancreas is not well understood and considering that there are no studies evaluating the expression profiles of the immune checkpoint molecules in pancreas disorders, we investigated the expression of HLA-G and -E in the pancreas specimens obtained from patients with T1D, T2D, alcoholic chronic pancreatitis (ACP) and idiopathic chronic pancreatitis (ICP).

2. Material and methods

2.1. Tissue specimens

Autopsy pancreas specimens were obtained from the archives of the Department of Pathology of the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, SP, Brazil. We studied 20 samples of histologically normal pancreas (HNP) obtained from subjects who died from non-pancreatic disorders, 30 samples of ACP, 10 samples of ICP, 10 samples of T1D and 30 samples of T2D patients. For each case, all available hematoxylin and eosin (H&E)-stained sections were reviewed to confirm the previous diagnosis and to select a representative block for immunostaining. The diagnosis of pancreas disorders was performed on the basis of established histological and immunohistochemical criteria. Cases with insufficient material or presenting evidence of any other pancreatic disorder were excluded. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee (n°12841/2018).

2.2. Immunohistochemistry assay for HLA-G and HLA-E

Formalin-fixed, paraffin-embedded pancreatic tissues were submitted to HLA-G and HLA-E immunohistochemical analysis. Three-μm-thick sections mounted on poly-l-lysine-coated slides were

deparaffinized, rehydrated, immersed in 10 mmol/L citrate buffer (pH 6.0), and submitted to heat-induced epitope retrieval for 45 minutes. The slides were rinsed with phosphate-buffered saline (PBS) and immersed into 3% hydrogen peroxide for 20 minutes to block endogenous peroxidase. Non-specific protein binding was blocked with normal serum (Vectastain Elite ABC Kit, Universal, Vector Laboratories, Burlingame, CA, USA) for 30 minutes. The sections were then incubated with monoclonal antibodies (mAbs) specific for HLA-G (MEM-G/02, mouse IgG1, dilution 1:100, EXBIO, Vestec, Czech Republic) or HLA-E (MEM-E/02, mouse IgG1, dilution 1:100, EXBIO). The selection of these mAbs was performed on the basis of: i) the available literature for the selection of HLA-G mAbs [21,22], ii) our previous experiences on immunohistochemistry (IHC) assay [23–26] and iii) the availability of commercial anti-HLA-G and anti-HLA-E mAbs for IHC on formalin-fixed, paraffin-embedded samples. The incubations were performed for two hours at room temperature (25 °C) in a humidity chamber. Following washes in PBS, a biotinylated pan-specific universal secondary antibody (Vector Laboratories) was applied for 30 minutes. The slides were incubated with the avidin–biotin–peroxidase complex (Vector Laboratories) for 30 minutes and incubated with diaminobenzidine (Vector Laboratories) for five minutes. The slides were counterstained with Harris hematoxylin, dehydrated and mounted with Permount (Biomeda, Foster City, CA, USA). Cytotrophoblast from first-trimester human placenta was used as an HLA-G and -E positive control. Negative controls were performed using rat liver tissue that does not express HLA-G or -E, and human HNP by omitting the primary antibody (Fig. 1).

The immunostaining was analyzed in three different areas of pancreas tissue (acini, islets and inflammatory infiltrate) and considered to be positive when a distinct brown membrane or cytoplasmic staining was homogenously present. The percentages of positive cells were obtained blindly at a representative high-power field (40x). For statistical purposes, the samples were scored as follows: − (no stained cells); + (weak staining, less than 25% of stained cells); + + (moderate staining, 25–50% of stained cells); and + + + (strong staining, more than 50% of stained cells).

2.3. Statistical analysis

HLA-G and HLA-E pancreas expression was compared between the different groups using the two tailed Fisher's exact test, performed by the GENEPOP 4.7 software [27]. A 5% level of significance ($\alpha = 0.05$) was considered for rejection of the null hypothesis.

3. Results

The expression profiles of HLA-G and HLA-E observed among the different studied groups are illustrated in Fig. 2.

3.1. HLA-G expression in pancreatic tissues

Initially, we evaluated the expression of HLA-G in each pancreatic condition, comparing the three different areas (acini, islets and inflammatory infiltrate) of the pancreas specimens (Table 1). In all HNP specimens, a strong HLA-G staining was observed in acini and islets, and HLA-G was not detected in the scanty mononuclear cells ($P = 0.0001$). In both cases of chronic pancreatitis (ACP and ICP), the HLA-G staining was lost in some acini and islets due to the replacement of the pancreatic parenchyma by fibrosis and fat. For most cases of ACP, HLA-G staining was weak in the remaining acini and islets, as well as in the inflammatory cells, and no statistical difference was observed between these different areas ($P = 0.9268$). Similarly, in ICP, a predominantly weak HLA-G staining was observed in the remaining acini, islets and in damage-associated inflammatory cells, and no statistical difference was detected when compared to other areas ($P = 0.8950$). Regarding T1D specimens, the HLA-G staining in islets was weaker when compared to acini and to inflammatory cells; however, without reaching significance ($P = 0.4447$). On the other hand, in T2D, we observed a significant increased HLA-G expression in acini, when compared to the weak staining in islets and in the few inflammatory cells ($P < 0.0001$) (Table 1).

The expression of HLA-G was also compared according to the cell type among the different pancreatic conditions (Table 1). Acini and islets from HNP presented a significantly higher HLA-G staining, when compared to all other conditions (*i.e.,* ACP, ICP, T1D and T2D, $P < 0.0020$ for all comparisons). Additionally, we observed that acini from T2D presented higher expression of HLA-G when compared to acini from ACP ($P = 0.0010$) and ICP ($P = 0.0070$). Since HNP did not exhibit inflammatory infiltrate, HLA-G expression in this area was primarily observed in pancreatic disorders, without reaching significance after comparing the groups.

3.2. HLA-E expression in pancreatic tissues

We also evaluated the expression of HLA-E in each pancreatic condition comparing the different cells of the pancreas tissue (Table 2). HLA-E expression was moderately observed in acini and islets in HNP, and was not observed in the low number of inflammatory cells ($P = 0.0001$). In chronic pancreatitis (ACP and ICP) and in T1D, the expression of HLA-E was also observed in different degrees in the remaining acini and islets, as well as in the few inflammatory cells; however, no significant differences were observed among the different areas ($P = 0.5590$ for ACP, $P = 0.9754$ for ICP and $P = 0.9755$ for T1D). On the other hand, in T2D, resembling to HLA-G, HLA-E staining was significantly increased in the acini, when compared to the weak staining in islets and in the few inflammatory cells ($P < 0.0001$) (Table 2).

The expression of HLA-E was also compared according to the cell type between different pancreatic conditions (Table 2). HLA-E was strongly stained in acini and islets in HNP, when compared to corresponding cell types from the other studied groups ($P \leq 0.0001$ for all comparisons). Furthermore, higher expression of HLA-E was observed in acini from T2D compared to acini from ACP ($P = 0.0011$). The expression of HLA-E was observed in the immune infiltrate of all pancreatic disorders evaluated in this series, and a significant increased HLA-E expression was observed in inflammatory cells from ICP when compared to inflammatory cells from T2D specimens ($P = 0.0441$).

4. Discussion

HLA-G has been increasingly studied in several chronic inflammatory, autoimmune and neoplastic disorders to understand the pathogenesis of these conditions, as well as to search for its possible use as a diagnostic marker, a prognostic factor or as a putative therapeutic tool [28]. Unlike HLA-G, the expression and function of HLA-E in physiological and pathological processes remain poorly studied; however, the molecule has also been evaluated in certain human disorders [29,30]. Since HLA-E has high affinity for the monomeric leader peptide derived from other HLA class I molecules, HLA-G molecule can induce the expression of HLA-E [17]. In this context, several studies have shown that HLA-G and HLA-E can co-operate in physiological conditions to establish an immunosuppressive microenvironment [20]. However, only few reports are available regarding the role of both HLA-G and HLA-E in inflammatory/autoimmune diseases, in which either an opposite or a synergistic role may occur in the course of these disorders [20].

Little attention has been devoted to the study of HLA-G expression in human pancreatic diseases, being primarily focused on pancreatic adenocarcinoma [31–35], which clinical relevance has been contradictory, since its expression has been associated with both worse tumor prognosis [33–35] and greater patient survival [32]. On the other hand, only the *HLA-E* gene profile has been studied in T1D pancreas

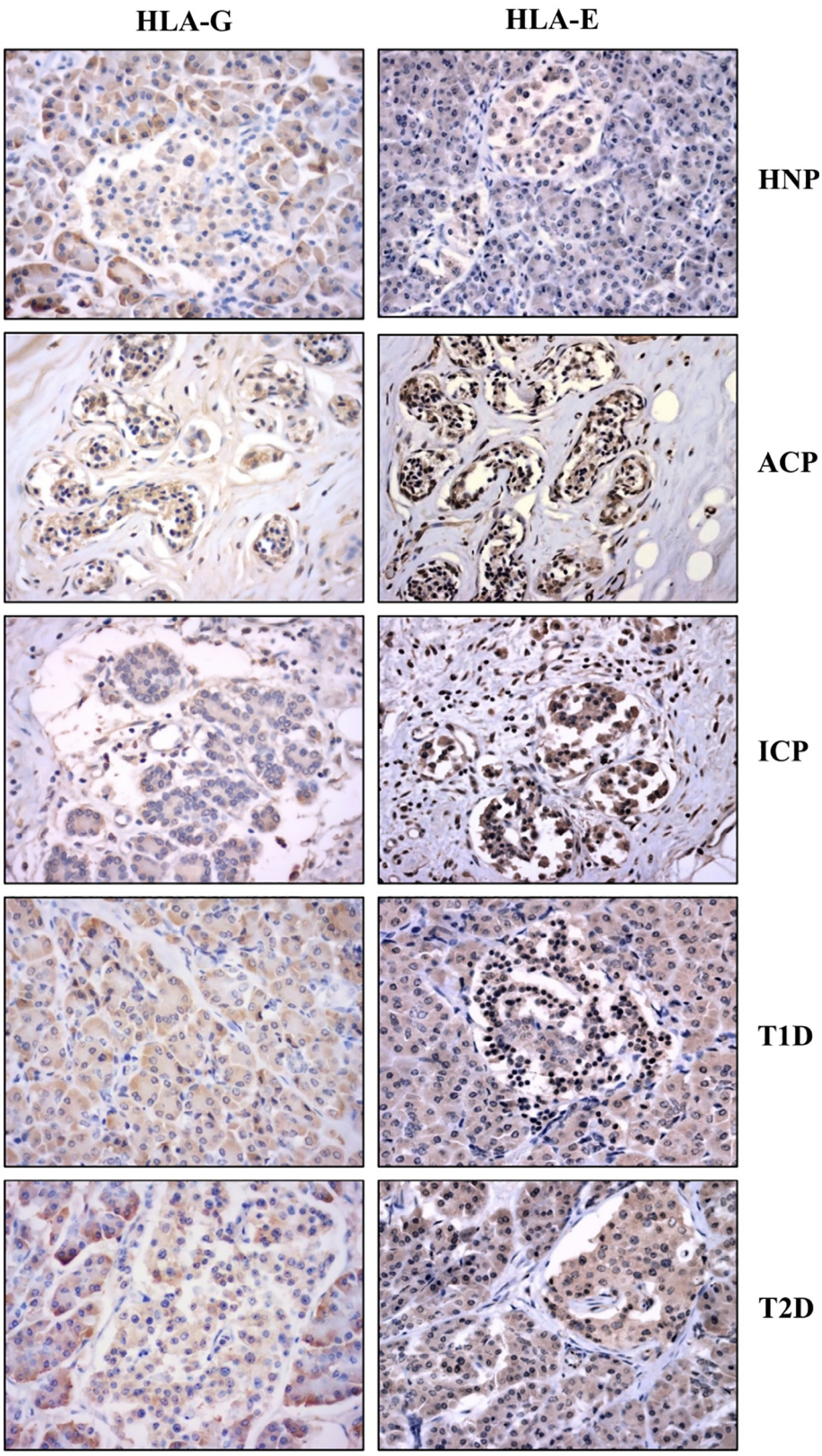

Fig. 2. Immunohistochemical staining for Human Antigen Leucocyte (HLA)-G and HLA-E. Representative photomicrographs (×200) of stained cells (brown staining) observed in histologically normal pancreas (HNP), alcoholic chronic pancreatitis (ACP), idiopathic chronic pancreatitis (ICP), type 1 diabetes (T1D) and type 2 diabetes (T2D).

Table 1
Human Antigen Leucocyte (HLA)-G expression in histologically normal pancreas (HNP), alcoholic chronic pancreatitis (ACP), idiopathic chronic pancreatitis (ICP), type 1 diabetes (T1D) and type 2 diabetes (T2D).

Condition	Area	HLA-G expression								Statistical analysis								
		−		+		++		+++		P^*	Comparison	$P^{\#}$	Comparison	$P^{\#}$	Comparison	$P^{\#}$	Comparison	$P^{\#}$
		n	%	n	%	n	%	n	%									
HNP (n = 20)	Acini	0	0.0	0	0.0	0	0.0	20	100	**0.0001**	–	–	–	–	–	–	–	–
	Islet	0	0.0	0	0.0	0	0.0	20	100		–	–	–	–	–	–	–	–
	Inflammation	20	100	0	0.0	0	0.0	0	0.0		–	–	–	–	–	–	–	–
ACP (n = 30)	Acini	15	50.0	6	20.0	5	16.7	4	13.3	0.9268	*vs.* HNP	**0.0001**	–	–	–	–	–	–
	Islet	18	60.0	5	16.7	5	16.7	2	6.7		*vs.* HNP	**0.0001**	–	–	–	–	–	–
	Inflammation	16	53.3	8	26.7	4	13.3	2	6.7		*vs.* HNP	**0.0013**	–	–	–	–	–	–
ICP (n = 10)	Acini	6	60.0	2	20.0	1	10.0	1	10.0	0.8950	*vs.* HNP	**0.0001**	*vs.* ACP	1.0000	–	–	–	–
	Islet	5	50.0	2	20.0	2	20.0	1	10.0		*vs.* HNP	**0.0001**	*vs.* ACP	1.0000	–	–	–	–
	Inflammation	4	40.0	1	10.0	2	20.0	3	30.0		*vs.* HNP	**0.0003**	*vs.* ACP	0.2105	–	–	–	–
T1D (n = 10)	Acini	1	10.0	2	20.0	3	30.0	4	40.0	0.4447	*vs.* HNP	**0.0003**	*vs.* ACP	0.0639	*vs.* ICP	0.1264	–	–
	Islet	6	60.0	1	10.0	2	20.0	1	10.0		*vs.* HNP	**0.0001**	*vs.* ACP	1.0000	*vs.* ICP	1.0000	–	–
	Inflammation	3	30.0	2	20.0	2	20.0	3	30.0		*vs.* HNP	**0.0001**	*vs.* ACP	0.2134	*vs.* ICP	1.0000	–	–
T2D (n = 30)	Acini	3	10.0	6	20.0	5	16.7	16	53.3	< **0.0001**	*vs.* HNP	**0.0017**	*vs.* ACP	**0.0010**	*vs.* ICP	**0.0070**	*vs.* T1D	0.8608
	Islet	18	60.0	5	16.7	5	16.7	2	6.7		*vs.* HNP	**0.0001**	*vs.* ACP	1.0000	*vs.* ICP	1.0000	*vs.* T1D	1.0000
	Inflammation	15	50.0	8	26.7	5	16.7	2	6.7		*vs.* HNP	**0.0006**	*vs.* ACP	1.0000	*vs.* ICP	0.2592	*vs.* T1D	0.2875

− (no staining); + (weak staining); ++ (moderate staining); and +++ (strong staining). P^* compares the three different cell types within each pancreatic condition. $P^{\#}$ compares a specific cell type between different pancreatic conditions, as indicated in the table. Comparisons were performed by Fisher's exact test. Significant values highlighted in bold ($P < 0.05$).

Table 2
Human Antigen Leucocyte (HLA)-E expression in histologically normal pancreas (HNP), alcoholic chronic pancreatitis (ACP), idiopathic chronic pancreatitis (ICP), type 1 diabetes (T1D) and type 2 diabetes (T2D).

Condition	Area	HLA-E expression								Statistical analysis								
		−		+		++		+++		P^*	Comparison	$P^{\#}$	Comparison	$P^{\#}$	Comparison	$P^{\#}$	Comparison	$P^{\#}$
		n	%	n	%	n	%	n	%									
HNP (n = 20)	Acini	0	0.0	0	0.0	20	100	0	0.0	**0.0001**	–	–	–	–	–	–	–	–
	Islet	0	0.0	0	0.0	20	100	0	0.0		–	–	–	–	–	–	–	–
	Inflammation	20	100	0	0.0	0	0.0	0	0.0		–	–	–	–	–	–	–	–
ACP (n = 30)	Acini	15	50.0	4	13.3	5	16.7	6	20.0	0.5590	*vs.* HNP	**0.0001**	–	–	–	–	–	–
	Islet	17	56.7	3	10.0	6	20.0	4	13.3		*vs.* HNP	**0.0001**	–	–	–	–	–	–
	Inflammation	18	60.0	7	23.3	3	10.0	2	6.7		*vs.* HNP	**0.0046**	–	–	–	–	–	–
ICP (n = 10)	Acini	1	10.0	3	40.0	2	20.0	4	40.0	0.9754	*vs.* HNP	**0.0001**	*vs.* ACP	0.0960	–	–	–	–
	Islet	3	30.0	2	20.0	2	20.0	3	30.0		*vs.* HNP	< **0.0001**	*vs.* ACP	0.4043	–	–	–	–
	Inflammation	2	20.0	2	20.0	3	30.0	3	30.0		*vs.* HNP	**0.0001**	*vs.* ACP	0.0336	–	–	–	–
T1D (n = 10)	Acini	1	10.0	3	30.0	3	30.0	3	30.0	0.9755	*vs.* HNP	< **0.0001**	*vs.* ACP	0.1054	*vs.* ICP	1.0000	–	–
	Islet	2	20.0	2	20.0	3	30.0	3	30.0		*vs.* HNP	< **0.0001**	*vs.* ACP	0.1891	*vs.* ICP	1.0000	–	–
	Inflammation	3	30.0	2	20.0	2	20.0	3	30.0		*vs.* HNP	**0.0001**	*vs.* ACP	0.1040	*vs.* ICP	0.1040	–	–
T2D (n = 30)	Acini	2	6.7	5	16.7	8	26.7	15	50.0	< **0.0001**	*vs.* HNP	**0.0001**	*vs.* ACP	**0.0011**	*vs.* ICP	0.7756	*vs.* T1D	0.5677
	Islet	11	36.7	8	26.7	6	20.0	5	16.7		*vs.* HNP	**0.0001**	*vs.* ACP	0.3172	*vs.* ICP	0.8545	*vs.* T1D	0.6189
	Inflammation	17	56.7	8	26.7	3	10.0	2	6.7		*vs.* HNP	**0.0018**	*vs.* ACP	1.0000	*vs.* ICP	**0.0441**	*vs.* T1D	0.1379

− (no staining); + (weak staining); ++ (moderate staining); and +++ (strong staining). P^* compares the three different cell types within each pancreatic condition. $P^{\#}$ compares a specific cell type between different pancreatic conditions, as indicated in the table. Comparisons were performed by Fisher's exact test. Significant values highlighted in bold ($P < 0.05$).

specimens obtained from living or deceased patients, showing increased gene expression when compared to healthy pancreas [36]. Nevertheless, the expression of both HLA-G and HLA-E molecules in distinct types of pancreatic diseases is poorly explored.

The constitutive expression of HLA-G in histologically normal pancreas (HNP) has been observed by us and by other authors [8], although HLA-G expression in HNP is controversial according to the Human Protein Atlas (HPA) [37], which reports no protein expression, but reports *HLA-G* mRNA detection according to three different RNA datasets, including HPA, Genotype-Tissue Expression consortium [38] and CAGE data from the FANTOM5 consortium [39]. Major discrepancies regarding protein expression may be due to: i) in HPA data, the HLA-G detection by immunohistochemistry was performed using the anti-HLA-G 4H84 mAb (sc-21799, Santa Cruz Biotechnology, Inc.) at a dilution of 1:3000, and ii) in our study, we used another mAb (MEM-G/02) at a dilution of 1:100.

HLA-G has important inhibitory properties over a broad spectrum of immunocompetent cells [40], thus its decreased expression in pancreatic tissue may influence the fine immune system cell modulation in inflammatory and autoimmune disorders [18]. Since the expression of HLA-E followed a closely similar pattern of HLA-G, this can be explained, at least in part, by the role of HLA-G on providing leader peptides for HLA-E [41]. Considering that HLA-E affinity to the CD94/NKG2A inhibitory receptor is six fold higher than its affinity to the CD94/NKG2C activating receptor [20], the decreased expression of HLA-E together with the decreased HLA-G may augment the activity of effector cells and decrease T_{regs} [42].

The constitutive pancreas expression of immune checkpoint molecules may protect islets and acini from destruction by cytotoxic cells in an environment highly rich in lytic enzymes, and the expression of these molecules, particularly of HLA-G, may also provide a tolerogenic milieu to protect these cells. As corollary, a decreased expression may propitiate tissue destruction and a diminished tolerogenic environment. Regarding T1D, cellular immunity plays a central role on the destruction of pancreatic islets, mainly mediated by auto reactive $CD4^+$ T-helper 1 cells (T_H1) and $CD8^+$ T cells and functional defects or decreased numbers of T_{regs} [1]. Besides islets destruction, impairment of pancreas exocrine function mediated by atrophy and fibrosis seems to be a frequent complication of T1D [43]. Although there is no direct immune-mediated destruction of the pancreatic tissue in T2D, fat and cell infiltration may contribute to the loss of pancreatic cells and insufficient pancreas function [44], explaining the decreased HLA-G and -E expression in islets and acini and the infiltration of inflammatory cells expressing these molecules. Noteworthy, among T2D specimens, we observed that acini presented higher HLA-G and HLA-E expression compared to islets and to inflammatory cells, indicating that islets may be more susceptible to damage induced by the decreased expression of both molecules.

The tissue damage observed in pancreatitis is mainly associated with the release of intracellular contents by damaged acini, which act as damage associated molecular patterns, triggering the inflammatory process. The innate immune response and rapid infiltration of neutrophils in the tissue, usually followed by recruitment of macrophages, amplify the response by releasing proinflammatory cytokines such as IL-1β, TNF-α e IL-6. During its chronic phase, $CD4^+$ T-helper 17 cells (T_H17), T_H1 and $CD8^+$ T cells predominate in the inflammatory infiltrate and are involved in the progression of the disease and fibrogenesis [5,45]. Although the course of the disease, type of complications and endocrine/exocrine impairments may differ in ACP and ICP [46], the expression profiles of HLA-G and -E and the expression of these molecules in infiltrating cells were closely similar. Noteworthy, ACP and ICP specimens exhibited decreased expression of HLA-G and -E in acini when compared to T2D specimens but not when compared to T1D specimens, corroborating the increased damage of acinar cells in pancreatitis. Taken together, the decreased HLA-G and HLA-E expression in islets and acini may reflect the pancreas damage, contributing to an increased activity of cytotoxic cells and decreased activity of T_{regs}.

Overall, we reported that HLA-G and HLA-E molecules are preferentially expressed in acini and islets of HNP, but their expression is significantly lost as far as acini and islets are destroyed or replaced by fibrosis or fat deposition. Therefore, irrespective of the major underlying mechanism associated with the pancreatic disorder, all groups exhibited different degrees of decreased HLA-G and HLA-E expression in islets and acini, together with an increased expression of these molecules in the inflammatory infiltrate. In addition to the destruction of islets and acini by chronic inflammatory, autoimmune and metabolic processes, specific features of each pancreatic disorder may also contribute to the expression of immune checkpoint molecules. For instance, microenvironment cytokines [40] and hypoxia [47] may also modulate the expression of HLA-G and -E molecules.

The restricted tissue expression of HLA-G and -E indicates a particular regulation of the genes, in which the regulatory region of *HLA-G* gene has been much more studied in comparison to *HLA-E*. At least four regulatory segments have been described at the 5′ upstream region (5′URR) of *HLA-G* gene that may modulate its expression: i) a proximal promoter (−1406 to −1) that is atypical when compared to the promoter of the classical class I genes, lacking nuclear factor κB (NF-κB) and interferon (IFN)-γ responsive elements [48], and exhibiting a locus control region (LCR), which is critical for spatiotemporal *HLA-G* transcription in placental trophoblasts [49]; ii) a distal promoter (−2635 to −1406) that may also be target of transcription factors related to immune system regulation [50]; iii) a negative regulator in a sequence about −4 kb upstream to the *HLA-G* translation starting point that overlapped with long interspersed elements (LINE1) [51] and iv) a cis-regulatory element situated −12 kb upstream of the *HLA-G* translation starting point exhibiting an enhancer activity [52]. These regulatory elements at several combinations may be responsible for the restricted HLA-G and -E tissue expression in both physiological and pathological conditions.

In conclusion, the decreased expression of HLA-G and -E in islets and acini together with the expression of these molecules in the inflammatory infiltrate were shared features among chronic inflammatory and autoimmune pancreatic disorders evaluated in this study. Although the pancreas cell damage appears to be the prominent mechanism associated with the decreased HLA-G and -E expression, specific factors associate with the pathogenesis of each disorder and genetic mechanisms may also contribute to the magnitude of the diminished expression. If, in the near future, pancreas biopsies become an available tool for diagnostic purposes, HLA-G and -E expression may be useful to evaluate the magnitude of islets and acini damage.

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.

Acknowledgements

We thank Flávia Tremeschin de Almeida Vieira and Sandra Silva Rodrigues dos Santos (Department of Medicine, Division of Clinical Immunology, Ribeirão Preto Medical School, University of São Paulo) for technical assistance.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. BCB is recipient of a PhD scholarship from São Paulo Research Foundation (FAPESP) [grant number #2015/26556-0].

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