Concise report

HLA-G and HLA-E in patients with juvenile idiopathic arthritis

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Abstract

Objective. To investigate the expression and release of HLA-G and HLA-E in JIA.

Methods. Soluble (s)HLA-G and HLA-E were measured in sera from 58 JIA patients and 54 healthy donors. Surface expression of HLA-G, HLA-E and immunoglobulin-like transcript (ILT)2 and ILT4, two receptors for HLA-G, was assessed on T, B cells and monocytes from peripheral blood (PB) and SF of 12 JIA patients and from PB of 12 controls.

Results. Serum sHLA-G concentration was significantly lower in patients than in controls. Both sHLA-G and sHLA-E were detected in SF and sHLA-E concentration in SF was higher in extended oligoarticular/polyarticular than in limited oligoarticular JIA. Patients compared with controls showed: (i) down-regulation of HLA-E and ILT2 expression on T cells; (ii) up-regulation of HLA-E expression on B cells and monocytes; and (iii) down-regulation of ILT4 expression on monocytes. Comparing JIA patients’ SF and PB we found: (i) up-regulation of HLA-E and ILT2 expression in T and B cells and monocytes; and (ii) down-regulation of ILT4 expression in monocytes. ILT4 was up-regulated in monocytes from oligoarticular extended/polyarticular compared with oligoarticular limited JIA.

Conclusions. A lower concentration of sHLA-G in sera may predispose to JIA, as observed for other autoimmune diseases. sHLA-E concentration in SF correlate with the number of affected joints. Higher ILT2 expression on SF cell populations compared with PB may be related to high sHLA-G concentration in SF. Higher HLA-E expression in SF than in PB cell populations may protect them from NK cytolysis.

Key words: Juvenile idiopathic arthritis, Human leucocyte antigen-G, Human leucocyte antigen-E.

Introduction

JIA comprises a heterogeneous group of chronic inflammatory arthritides with unknown aetiology. The JIA classification was developed by the ILAR [1]. The most frequent forms of JIA are oligoarticular and polyarticular JIA. Oligoarticular JIA affects four or fewer joints during the first 6 months of disease (26–56% of patients). It is characterized by early disease onset, asymmetric arthritis, association with peculiar HLA genotypes and presence of ANAs. The majority of these patients present a persistent oligoarticular course, with limited affected joints, favourable outcome and high frequency of self-remission. Conversely, 30% of these patients progress towards extended oligoarticular JIA that involves five or more joints [2].

RF-negative polyarthritis affects five or more joints during the first 6 months of disease in the absence of immunoglobulin (Ig)M RF (11–28% of JIA patients). A small proportion of JIA patients present RF-positive polyarthritis, characterized by symmetric involvement of more than four joints, with an erosive course similar to adult RA. Other rarer forms of JIA are systemic arthritis, PsA and enthesitis-related arthritis [2].

A common JIA feature is the presence of synovial lymphocytic infiltrates that play a role in JIA pathogenesis through the release of pro-inflammatory cytokines and other soluble factors. Both T and B cells are recruited in JIA lesions by chemokine gradients, through the
expression of a peculiar set of chemokine receptors [3, 4]. T cells are mostly autoreactive cells, specific for partially defined antigens [5], whereas infiltrating B cells are either switch-memory B cells that express high levels of CD86 and act as antigen-presenting cells, or IgG-secreting plasma blasts [3].

JIA patients also display expansion and activation of monocytes that secrete pro-inflammatory cytokines, and show enhanced proteolytic activity. Moreover, JIA patients' serum contains macrophage migration inhibitory factor that may drive monocyte activation [6].

An association between JIA and RA and a 14-bp insertion/deletion polymorphism of the HLA-Ib molecule HLA-G has been recently demonstrated [7]. Moreover, soluble (s)HLA-G concentration in sera from patients with RA [7] is lower than in healthy subjects, as observed in other autoimmune rheumatic diseases [8].

HLA-class Ib subfamily comprises HLA-G, HLA-E, HLA-F and HLA-H. These molecules display a lower degree of polymorphism than highly polymorphic classical HLA-class Ia molecules, and are expressed not only as surface molecules on different cell populations, but also as soluble moieties in biological fluids [9]. In contrast to classical HLA molecules, which are mainly involved in the presentation of peptides for recognition by TCRs on T cells, as well as in the interaction with killer Ig-like receptors on NK cells, HLA-Ib molecules display several immunoregulatory properties.

Among HLA-class Ib molecules, HLA-G and HLA-E are the best characterized molecules. The physiological role of HLA-G is the establishment of a tolerogenic environment at the maternal/fetal interface by abrogating the maternal immune response against fetal tissue [10]. HLA-G exerts different immunoregulatory features (i.e. inhibition of cytotoxic T lymphocytes (CTL) and NK lysis, induction of apoptosis of T and NK cells, inhibition of T-cell chemo-taxis, modulation of pro-angiogenic factor release by NK cells) by interacting with four inhibitory receptors: immunoglobulin-like transcript (ILT)2 (on T, B, NK cells and monocytes), ILT4 (on monocytes), KIR2DL4 (on NK cells) and CD160 (on T, NK and endothelial cells) [11].

The main function of HLA-E is to present peptides derived from the leader sequence of HLA-class I molecules to NK cells through the interaction with CD94/NKG2A, thus allowing NK cells to monitor the level of HLA-class I expression. The interaction between HLA-E/peptide complex and CD94/NKG2A inhibits NK cell lysis [12].

With this background we have here investigated the expression of HLA-G and HLA-E molecules on cell populations from peripheral blood (PB) and SF of JIA patients, compared with healthy subjects. The presence of soluble HLA-G and HLA-E in sera and the JIA microenvironment, i.e. SF, has also been analysed.

Methods

Patients

This study was approved by the ethics committee of the G. Gaslini Institute, Genoa, Italy. PB and SF from 58 JIA patients (46 females and 12 males, age range 1.9–22.6 years, mean 10 years) and PB from 54 age- and sex-matched healthy controls were obtained following informed consent of patients' parents or legal guardians according to the Declaration of Helsinki. JIA individuals were classified according to ILAR Durban criteria [1]. Twenty-nine patients had limited oligoarticular JIA (21 females and 8 males, age range 2.1–19.6 years, mean 9.8 years), while the remaining 29 had extended oligoarticular or polyarticular JIA (25 females and 4 males, age range 1.9–22.6 years, mean 9.7 years). All samples were collected from untreated patients at diagnosis.

Mononuclear cells (MNCs) were isolated by Ficoll–Hypaque density gradients (Sigma Chemical Company, St Louis, MO, USA) from PB or SF of 12 patients with JIA (6 with limited oligoarticular JIA, 3 males and 3 females, and 6 with extended oligoarticular/polyarticular JIA, 3 males and 3 females) and from PB of 12 age- and sex-matched healthy controls.

ELISA

Concentration of sHLA-G or sHLA-E was evaluated on JIA patients’ sera and SF, or sera from controls. ELISA was performed as previously described for sHLA-G [13].

For sHLA-E detection, 3D12 specific mAb (kindly donated by Dr Geraghty; 10 μg/ml) was used as capture reagent. A standard curve was generated using serial dilutions of total extract from normal PBMCs, and it was expressed in arbitrary units (U) (1 U/ml equaled the amount of HLA-E in 1 μg/ml normal PBMCN lysate). Each sample was tested in duplicate.

Flow cytometry

Surface HLA-G, HLA-E, ILT2 and ILT4 expression was evaluated as previously described [13] on PBMCs and SFMCs from 12 JIA patients and PBMCs from 12 age- and sex-matched controls. The following mAbs were used: MEM-G9 mAb specific for HLA-G (Exbio, Vestec, Czechoslovakia), 3D12 mAb specific for HLA-E, F278 mAb specific for ILT2 (kindly donated by Dr Daniela Pende), anti-ILT4 PE mAb (Becton Dickinson, CA, USA). Goat F(ab)2 anti-mouse IgG1 PE conjugated was used as secondary antibody (Southern Biotech, Birmingham, AL, USA).

HLA-G, HLA-E ILT2 and ILT4 expression was evaluated gating on CD3+, CD19+ and CD14+ cells, using anti-CD3-PC7 (Beckman Coulter, Brea, CA, USA), anti-CD19-FITC and anti-CD14-APC mAbs (Immunotools, Friesoythe, Germany). Data were acquired and analysed using a Gallios cytometer and Kaluza Software (Beckman Coulter). Data are expressed as mean relative fluorescence intensity (MRFI).

Statistical analysis

Data were compared using t-test. Statistical analyses were performed using Prism Software 3.02 (GraphPad Software Inc., La Jolla, CA, USA).
Results

We first investigated the presence of sHLA-G and sHLA-E in paired SF and serum samples from two groups of JIA patients with limited oligoarticular and extended oligoarticular/polyarticular JIA. Comparative analysis was performed on age- and sex-matched healthy donors.

sHLA-G concentration was lower in sera from JIA patients than in those from controls [Fig. 1A; 8.3 (7.45) vs 16.67 (32.61) ng/ml, P = 0.03], without any difference between limited oligoarticular or extended oligoarticular/polyarticular JIA. In addition, sHLA-G was detected in SF from JIA patients [concentration 19.75 (16.16) ng/ml], with similar concentrations in limited oligoarticular [19.48 (18.44) ng/ml] and extended oligoarticular/polyarticular JIA [20 (14.13) ng/ml] (Fig. 1A).

In contrast, serum sHLA-E concentration was similar in JIA patients and controls, and in the two groups of JIA patients (Fig. 1B). sHLA-E was detected in SF from JIA patients [37.37 (36.06) U/ml], with higher concentration in extended oligoarticular/polyarticular [47.4 (40.85) U/ml] than in limited oligoarticular JIA cases [26.31 (26.41) U/ml, P = 0.01] (Fig. 1B).

Analysis of HLA-G and HLA-E surface expression was performed on T, B cells and monocytes from PB and SF of 12 JIA patients and from PB of 12 healthy donors.

Surface HLA-G expression on PB cell populations was similar in patients and controls. Moreover, no differences in surface HLA-G expression were detected comparing PB and SF cell populations from JIA patients, and comparing PB and SF cell populations from limited oligoarticular and extended oligoarticular/polyarticular patients (data not shown).

HLA-E expression on T cells was significantly lower in patients than controls [Fig. 2A, MRFI 2.05 (0.34) vs 5.42 (1.91), P < 0.0001], whereas B cells and monocytes expressed significantly higher levels of HLA-E in patients than controls [Fig. 2B, MRFI 1.84 (2.04) vs 4.77 (2.34), P = 0.004 and Fig. 2C, MRFI 1.5 vs 2.32 (0.77), P = 0.0016].

Furthermore, HLA-E expression was significantly higher in patients' SF than in PB T cells [Fig. 2A, MRFI 2.8 (0.69) vs 2 (0.34), P = 0.0014], B cells [Fig. 2B, MRFI 19.7 (13.7) vs 7.2 (1.8), P = 0.0025] and monocytes [Fig. 2C, MRFI 6.5 (3) vs 4.4 (1.5), P = 0.01]. HLA-E expression was similar in SF and PB cell populations between the two groups of patients (Fig. 2A–C).

Next, we analysed ILT2 and ILT4 surface expression on all the same cell populations from PB and SF of JIA patients and PB and controls. ILT2 expression on T cells was lower in patients than controls [Fig. 2D, MRFI 1.58 (0.39) vs 3.63 (1.45), P < 0.0001], whereas no differences were detected for B cell and monocyte expression of ILT2.

Moreover, ILT2 expression was higher in SF than in PB T cells [Fig. 2D, MRFI 2.34 (0.47) vs 1.58 (0.39), P = 0.0002], B cells [Fig. 2E, MRFI 58.49 (45.46) vs 22.91 (6.25), P = 0.0062] and monocytes [Fig. 2F, MRFI 76.86 (46.12) vs 47.09 (32.23), P = 0.04], but it was similar in SF and PB cell populations between the two groups of patients (Fig. 2D–F).

ILT4 expression, which is restricted to monocytes, was lower in patients than controls [Fig. 2G, MRFI 4.86 (1.96) vs 7.84 (3.83), P = 0.03], and higher in oligoarticular extended/polyarticular than oligoarticular limited cases [Fig. 2G, MRFI 6.13 (1.55) vs 4 (1.82), P = 0.04]. Moreover, ILT4 expression on patients' monocytes was lower in SF than in PB (Fig. 2G, MRFI 3.25 (1) vs 4.86 (1.96), P = 0.01).

Discussion

In this study, we demonstrate for the first time that HLA-G and HLA-E may be implicated in JIA pathogenesis. We show that sHLA-G concentration is lower in sera from JIA patients than from healthy donors. Several studies have previously demonstrated that serum sHLA-G levels are decreased in RA and other inflammatory rheumatic diseases [8], thus suggesting that lower serum sHLA-G concentration in sera correlates with susceptibility to autoimmune diseases. Since HLA-G molecules perform different immunoregulatory functions, such as inhibition of NK cells and CTL cytotoxicity, inhibition of CD4+ T-cell proliferation, induction of apoptosis of T, B and NK cells and impairment of T-cell chemotaxis [11, 14], we speculate that decreased sHLA-G serum concentration may lead to a chronic activation of inflammatory cells. This may indeed contribute to the development of autoimmune diseases [15].

A high concentration of sHLA-G molecules has been detected in SF. The high release of sHLA-G in the inflamed synovium may be related to the recruitment of activated monocytes and/or other myeloid cells, which are able to secrete high amounts of sHLA-G. Accordingly, the migration of a high proportion of sHLA-G secreting cells from the PB to the inflamed synovium may contribute to the decreased levels of sHLA-G observed in sera of JIA patients.

We also demonstrated that T and B cells and monocytes from SF of JIA patients display higher expression of the HLA-G ligand ILT2 than PB counterparts. ILT2 up-regulation and high concentration of sHLA-G in SF may be related, since previous studies have demonstrated that sHLA-G can up-regulate ILT2 expression [16]. Conversely, ILT4 expression was higher in PB than in SF monocytes. However, no information is available in the literature about a possible modulation of ILT4 expression induced by sHLA-G.

sHLA-E has been detected in sera and SF from JIA patients. However, serum HLA-E levels were similar in patients and controls. The function of sHLA-E is still debated. Recently, Coupel et al. [17] have demonstrated that sHLA-E is released by activated endothelial cells. Since endothelial cell activation is a common feature in inflamed synovium [18], we can hypothesize that sHLA-E in SF may be secreted predominantly by the latter cells. This hypothesis was supported by the finding that sHLA-E concentration was significantly higher in extended oligoarticular/polyarticular than in limited oligoarticular JIA, suggesting a correlation between sHLA-E levels in SF and the number of affected joints in JIA patients.
Fig. 1 sHLA-G (A) and sHLA-E (B) concentration was evaluated by ELISA in sera from JIA patients (n = 58) and age- and sex-matched healthy subjects [healthy donors (HD), n = 54]. Patients were subsequently analysed as limited oligoarticular JIA (n = 29) vs extended oligoarticular/polyarticular JIA (n = 29). Next, sHLA-G concentration was evaluated by ELISA on SF from total JIA patients (n = 58), limited oligoarticular JIA (n = 29) and extended oligoarticular/polyarticular JIA (n = 29). Data are expressed in scatter plots as ng/ml HLA-G and arbitrary units (U)/ml HLA-E. Horizontal bars indicated mean values. P-value is indicated when difference is statistically significant.
Fig. 2. Surface expression of HLA-E (A–C), ILT2 (D–F) and ILT4 (G) was evaluated by flow cytometry on T cells (A and D), B cells (B and E) and monocytes (C, F and G) from PB of JIA patients (n = 12) and age- and sex-matched healthy donors (HD, n = 14), and from SF of patients. Patients were subsequently analysed as limited oligoarticular JIA (n = 6) vs extended oligoarticular/polyarticular JIA (n = 6). Data are expressed in scatter plots as MRFI (mean fluorescence intensity obtained with specific mAb/mean fluorescence intensity obtained with irrelevant isotype-matched mAb). Horizontal bars indicate mean values. P-value is indicated when difference is statistically significant.
Surface HLA-G expression was not altered in JIA patients. Conversely, with the exception of T cells, HLA-E expression was higher on cells from patients than controls. Furthermore, comparing PB cells of patients with SF counterparts, we found an up-regulation of HLA-E expression in the latter cells.

HLA-E, and its homologue Qa-1 in mice, inhibits NK cell cytolysis through the interaction with CD94/NKG2A [19]. A recent work demonstrated that, in a mouse model of autoimmune encephalomyelitis, antibody-dependent blockade of Qa-1–CD94/NKG2A interaction resulted in NK-dependent elimination of activated autoreactive T cells, and amelioration of disease [20]. We can hypothesize that HLA-E up-regulation on autoreactive cells in SF may protect them from NK cell lysis, leading to a persistent autoimmune response in the synovium.

In conclusion, both HLA-G and HLA-E may be related to JIA onset. HLA-G is more important as a soluble molecule in biological fluids, where it may control immune cell activation. In JIA, similarly to other autoimmune diseases, this function is impaired, since sHLA-G concentration is lower. Conversely, sHLA-E may be important in inflamed synovium, since sHLA-E concentration in SF is high (and probably related to endothelial cell activation), and sHLA-E levels in SF correlated with disease stage. Finally, up-regulation of surface HLA-E expression on SF cells may impair the control of autoreactive cells by NK cells leading to more severe tissue damage.

**Rheumatology key messages**

- Lower sHLA-G serum levels detected in JIA patients may be associated with JIA development.
- sHLA-E levels detected in patients’ SF correlated with the number of affected joints.
- HLA-E expression is higher on SF than PB cells, and may impair NK cytotoxicity.

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