Maturation of antigen-presenting cells is compromised in HLA-G transgenic mice

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Abstract

The human MHC class Ib antigen HLA-G is thought to regulate maternal immune responses during pregnancy. Here we show that expression of HLA-G in transgenic mice diminished cellular immunity by inhibiting maturation of myelomonocytic cells into functional antigen-presenting cells (APC). Skin allografts applied to HLA-G transgenic mice survived longer and resultant T cell responses were less potent compared to control mice. T cells from HLA-G mice responded normally to allogeneic APC and immunohistological analyses of spleen revealed no marked abnormalities. However, spontaneous outgrowths of myeloid cells were observed when bone marrow or splenocytes from HLA-G mice were cultured in vitro, but functionally competent APC did not develop spontaneously in bone marrow cultures supplemented with granulocyte macrophage colony stimulating factor (GM-CSF). Addition of lipopolysaccharide (LPS) to GM-CSF-derived bone marrow cultures rescued APC maturation. Studies using HLA-G tetrameric reagents revealed that HLA-G-specific binding activity was associated with CD11c⁺ myelomonocytic cells, while binding to lymphoid and NK cell subsets was undetectable. These data show that spontaneous maturation of functionally competent dendritic cells (DC) is compromised in HLA-G mice. We hypothesize that HLA-G inhibits maturation of DC via receptor-mediated interactions with myelomonocytic precursors, which render immature DC precursors unable to receive signals from activated T cells.

Introduction

The human MHC class Ib polypeptide HLA-G is expressed at the maternal–fetal interface during pregnancy (1–3). This unusual pattern of MHC expression has prompted speculation that cells expressing HLA-G (HLA-G⁺ cells) help protect the developing fetus from attack by maternal T cells capable of recognizing paternally inherited polymorphic MHC molecules as alloantigens. Other investigators have speculated that HLA-G⁺ cells might inhibit NK cell-mediated cellular immunity directed against cells expressing low or undetectable levels of classical HLA class I molecules (4,5). This would explain why NK cells do not attack human trophoblast cells, which do not express HLA-A, -B or -C molecules, during pregnancy. A third possibility is that HLA-G⁺ cells might present peptides derived from protein components of pathogens that infect trophoblast cells, eliciting maternal T cell immunity that protects the fetus (6,7). However, it is unclear how an essentially monomorphic MHC I molecule expressed almost exclusively at the maternal–fetal interface could select thymocytes and regulate peripheral cellular immune responses. Investigation of these potential roles for HLA-G⁺ cells during pregnancy has been hampered due to an as-yet undefined rodent HLA-G homologue and the consequent lack of a murine model for experimental studies in vivo.

Experimental evidence that HLA-G⁺ cells suppress T and NK cell responses has been obtained from in vitro studies (8–10). Several investigators have demonstrated that transfected HLA-G⁺ cells suppress T and NK cell effector responses (8–11). Inhibition of T cell responsiveness was antigen non-specific, as HLA-G-transfected cells suppressed T cell responses directed at antigens expressed on third-party stimulator cells (12,13). However, the ‘direct role’ of HLA-G in inhibiting NK cells is still controversial. Recent studies demonstrated an ‘indirect’ pathway in which increased surface expression of HLA-E associated with HLA-G leader
sequence peptides rather than HLA-G molecules provided signals to NK inhibitory receptors (14–16). These results support the hypothesis that HLA-G+ cells suppress T and NK responses in the local tissue microenvironment at the maternal-fetal interface. Nevertheless, the mechanisms by which cells expressing HLA-G deliver molecular signals that moderate T and NK cell responsiveness remain obscure, and further progress depends on better definition of the nature and functional consequences of HLA-G–receptor interactions involving T and NK cells and HLA-G+ cells.

In this report we demonstrate that myelomonocytic development, differentiation and function is abnormal in transgenic mice that express HLA-G (HLA-G mice). We show that development of mature antigen-presenting cells (APC) was compromised, providing a mechanistic explanation for decreased potency of T cell responses in HLA-G mice.

**Methods**

**Mice**

CBA/Ca (H-2k), C57BL/6 (B6, H-2b), BALB/c (H-2d) and C3H/HeJ (H-2f) mice were bred and maintained in a pathogen-free facility at the Medical College of Georgia. Transgenic HLA-G mice (on a CBA/Ca background) carry a recombinant H-2Kb/HLA-G gene expressed in all cells and have been described previously (17). Two independent lineages of homozygous transgenic mice (designated T1 and T3 mice) were established by mating sibling transgenic mice. Homozygous mice from both lines were used for these experiments and results were identical.

**Skin grafts**

Skin grafting was performed by the method of Billingham and Medawar (18). Skin (1.0 cm²) from the tail of donor mice was grafted onto the flanks of recipient mice (8–12 weeks old). Grafts were covered with gauze and plaster that were removed on day 10. Grafts were inspected daily until rejection. Rejection was scored when >60% of grafted tissue was necrotic and reduced in size. Recipients that were heterozygous or homozygous for the HLA-G transgene rejected skin allografts over similarly prolonged time periods compared to non-transgenic recipients.

**Generation of dendritic cells (DC)**

DC were generated from bone marrow by the method of Inaba et al. (19). In brief, bone marrow was flushed from the long bones of the limbs with air-buffered wash medium (IMDM) and centrifuged. The cell suspension was adjusted to 5×10⁶/ml in culture medium containing 25 ng/ml murine granulocyte macrophage colony stimulating factor (GM-CSF) (kindly provided by Dr B. Stockinger), IMDM medium (Life Technologies, Gaithersburg, MD) supplemented with 10% (v/v) FCS, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin was used for cell culture. On day 3 of culture non-adherent cells were gently removed and fresh GM-CSF-containing medium was added. On day 10, non-adherent cells (DC) were collected for analysis. More than 95% of cells produced in this way stained with the murine pan-DC marker, anti-CD11c mAb (PharMingen, San Diego, CA). In some experiments, additional maturation of DC was generated by the treatment on day 6 (immature DC) with various concentrations of lipopolysaccharide (LPS; Sigma, St Louis, MO). After 48 h, DC were assayed for their ability to stimulate T cell proliferation or were subjected to flow cytometric analyses.

**Cytotoxicity assay**

Effector cells were generated in mixed lymphocyte reaction and tested in a standard 51Cr-release assay. Briefly, spleen cells (5×10⁶ cells/ml) from naive HLA-G mice or mice that had rejected allogeneic skin grafts were co-cultured with irradiated allogeneic splenocytes (5×10⁶ cells/ml) for 5 days. Cytotoxicity tests were performed in triplicate on round-bottom microtiter plates (Falcon) at E:T ratios of 30:1, 10:1, 3:1 and 1:1. Targets cells were incubated for 90 min at 37°C with 100 μCi ⁵¹Cr (Amersham, Piscataway, NJ) before being mixed with effector cells. After 4 h at 37°C 25 μl of supernatant was collected and counted. NK cell purity, as assessed by flow cytometry, was determined by gating on CD11c, CD11b, CD40, CD45R/B220, CD80 (B7-1), CD86 (B7-2), DX5 (pan-NK cells), H-2E and H-2A (MHC class II alloantigens) were obtained from PharMingen. F4/80 mAb

**Antibodies and flow cytometry**

mAb to the following antigens were used. CD3, CD4, CD8, CD11c, CD11b, CD40, CD45R/B220, CD80 (B-7,1), CD86 (B-7,2), DX5 (pan-NK cells), H-2E and H-2A (MHC class II alloantigens) were obtained from PharMingen. F4/80 mAb
were purchased from Caltag (Burlingame, CA). Isotype control mAb included rat IgG2a and IgG2b, and hamster IgG and IgM (PharMingen). For fluorescent cell staining, mAb were used at 1 μg/10^6 cells. To analyze stained cells, 25,000 events were collected on a FACSCalibur calibrated with CaliBRITE beads using CellQuest software for collection and subsequent analyses (Becton Dickinson, Mountain View, CA). Analyses were carried out on live cells (>95%) as defined by forward and side angle scatter.

**Immunohistochemistry**

Frozen spleen fragments were cut, air-dried, fixed in acetone and rehydrated in Tris-buffered saline (TBS) containing 0.05% Tween 20. Endogenous peroxidase was blocked with 0.3% H2O2. For CD11b staining, sections were incubated with purified anti-CD11b (PharMingen), containing 15 μg/ml of rat IgG or an isotype-matched mAb control followed by biotin-conjugated goat F(ab')2 anti-rat IgG (Jackson ImmunoResearch, West Grove, PA) and horseradish peroxidase (HRP)-streptavidin (Biogenex, San Ramon, CA). HRP localization was revealed using a metal-enhanced diamino-benzidine substrate (Pierce, Rockford, IL), resulting in brown/black staining. Anti-CD11c purified mAb (PharMingen) containing 25 μg/ml hamster IgG was subsequently added, followed by biotin-conjugated goat F(ab')2 anti-hamster IgG cross-absorbed against rat and mouse serum proteins (Jackson ImmunoResearch) and HRP–streptavidin.

**HLA-G tetramer**

HLA-G tetramer was generated as previously described (20). Using HLA-G cDNA as a template, extracellular domains (amino acids 1–276) of HLA-G1 were amplified by PCR and cloned into a pET 23 vector (Novagen, Madison, WI) containing a BirA recognition and biotinylation site in-frame at the C-terminus. The HLA-G tetramer was created using synthetic peptide RIIPRHLQL (Molecular Biology Core Facility, Medical College of Georgia), previously shown bound to HLA-G (6). The peptide sequence is found in a region of histone H1, which is conserved in mice and humans (21). Dilutions for flow cytometry staining contained 10 μg/ml of refolded HLA-G/β2-microglobulin. As an irrelevant tetramer we used H-2Db tetramer refolded with NP 366–374 peptide derived from influenza A virus (kindly provided by Dr D. Moskofidis).

**Results**

**HLA-G mice exhibited reduced cellular immune responsiveness**

Two independent lineages of transgenic mice (designated T1 and T3 mice) were generated that expressed HLA-G under the control of promoter elements derived from the murine H-2Kβ gene. Previously, we reported that HLA-G expression occurs on most, if not all, cell types tested in HLA-G mice, and demonstrated that HLA-G behaves as a major transplantation antigen and restriction element for peptide presentation in mice (17).

HLA-G mice exhibited substantially reduced abilities to reject allogeneic tail skin grafts compared to non-transgenic CBA mice (Table 1). Mean survival times of skin grafts fully mis-matched at major and multiple minor histocompatibility loci (BALB/c and B6) or mis-matched at multiple minor histocompatibility loci only (C3H/HeJ) were prolonged. In addition, mean graft survival times on recipients homozygous or heterozygous for the HLA-G transgene were comparable. However, all grafts were rejected eventually, indicating that graft alloantigen-specific cellular immune responses were not completely suppressed in HLA-G mice.

Cytolytic T cell responses generated from HLA-G mice that had rejected skin allografts were less potent than when cytolytic T cells originated from grafted CBA mice (Fig. 1). Cytotoxic T cell activities directed against BALB/c and B6 target cells were significantly lower from allograft primed HLA-G mice than non-transgenic CBA mice. Thus, reduced responsiveness to skin allografts manifested in all HLA-G mice and correlated with reduced potency of cytolytic T cell responses. Moreover, significant lower cytolytic T cell responses were obtained in HLA-G mice infected with LCMV than in wild-type mice (Fig. 1C).

**Myelomonocytic cell development was abnormal in HLA-G mice**

Immunohistologic and flow cytometric analyses were conducted to elucidate why allograft-specific T cell responses were less potent in HLA-G mice. Spleen histology appeared similar in HLA-G and CBA mice, except that marginal zones were moderately expanded by populations of large cells (Fig. 2A and B). Modest increases in extramedullary hematopoiesis were observed in HLA-G mice relative to CBA controls. Immunohistologic analyses revealed focally extensive increases in CD11c+ cells in marginal zones of HLA-G spleen, but overall CD11c staining was comparable with CBA spleen (Fig. 2C and D). CD11b staining was found with regularity throughout the red pulp, and was essentially identical in HLA-G and CBA spleen (Fig. 2E and F). Flow cytometric analyses of splenocytes from HLA-G and CBA mice stained with antibodies specific for lymphoid (CD3, CD4, CD8 and B220), myeloid (F4/80) and NK (DX5) markers yielded similar staining patterns (Fig. 3). Thus, histologic and immunohistologic analyses revealed no striking distinctions between HLA-G and CBA mice, except that slightly increased numbers of CD11c+ cells were present in periarteriolar sheaths surrounding splenic white pulp areas in HLA-G mice.

However, evidence that development of myelomonocytic cell lineages was abnormal in HLA-G mice was obtained when bone marrow samples from HLA-G mice were cultured in the absence of exogenous growth factors (Fig. 2G and H). Under these conditions, monolayers of adherent cells grew rapidly and spontaneously when bone marrow originated from HLA-G mice; flow cytometric analyses showed that cellular outgrowths were CD11b+ and expressed low levels of F4/80 (data not shown). In contrast, few cells from CBA bone marrow survived under these culture conditions. After culture of HLA-G bone marrow, media did not contain detectable macrophage colony stimulating factor activity when assessed in a sensitive bioassay (22). Spontaneous outgrowths of adherent cells were also observed when splenocytes from HLA-G mice were placed in culture without added growth factors (data not shown). These data suggested that development and
HLA-G mediated immunodeficiency

Table 1. Allogeneic skin rejection is prolonged on HLA-G transgenic recipient mice

<table>
<thead>
<tr>
<th>Graft donor</th>
<th>Graft recipient</th>
<th>No. rejected/ no. grafted</th>
<th>Graft survival time (days)</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>CBA</td>
<td>6/6</td>
<td>13–18</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td>HLA-G</td>
<td>7/7</td>
<td>20–26</td>
<td>0.0023</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>CBA</td>
<td>7/7</td>
<td>10–13</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>HLA-G</td>
<td>8/8</td>
<td>21–24</td>
<td>0.0006</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>CBA</td>
<td>6/6</td>
<td>22–42</td>
<td></td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>HLA-G</td>
<td>9/9</td>
<td>58–117</td>
<td>0.0007</td>
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^aCompared with CBA graft recipients (Mann–Whitney test).

The results shown are the mean survival time ± SEM of values obtained in each group.

Fig. 1. Cytolytic T cell responses. Splenocytes from HLA-G (●) and CBA (○) mice that had rejected skin grafts from BALB/c (A) or C57Bl/6 (B) mice were co-cultured with irradiated splenocytes from BALB/c and C57BL/6 mice. T cell blasts were used as targets (A and B). Splenocytes from HLA-G (●) and CBA (○) mice that had been infected with LCMV were tested on day 8 for ex vivo cytotoxic T lymphocyte activity on infected L929 target cells (C). Cytolytic activity was assessed as described in Methods. Data shown is representative of at least four separate experiments.

differentiation of myelomonocytic cell lineages was abnormal in HLA-G mice.

T cells from HLA-G mice responded normally to allogenic APC

T cells from HLA-G mice responded normally when co-cultured with allogeneic APC (Fig. 4A and B). APC were mature DC generated from allogeneic (BALB/c and B6) bone marrow cultured with GM-CSF (19). APC from BALB/c and B6 mice elicited T cell proliferative responses of comparable potency when responder T cells originated from HLA-G or CBA mice. These data demonstrated that T cells from HLA-G mice responded normally when challenged with allogeneic APC in vitro. However, reduced levels of NK cytolytic activity from HLA-G mice were detected using YAC-1 target cells (Fig. 4C). Thus, T cells from HLA-G mice responded normally when stimulated by potent APC in vitro while NK activity measured ex vivo was lower. Since T cells, but not NK cells, are essential for tissue allograft rejection, these data suggested that depressed T cell responsiveness in HLA-G mice was not the cause of their reduced ability to reject skin allografts.

Spontaneous maturation of APC was defective in HLA-G mice

Next, we examined DC maturation by culturing bone marrow from HLA-G mice in the presence of GM-CSF and testing whether elicited cells provoked T cell proliferation. After 10 days the microscopic appearance of cultured bone marrow cells from HLA-G and CBA mice were strikingly different (Fig. 5A and B). Clusters of cells with dendritic morphology were prominent in cultures from CBA mice but they did not develop in cultures from HLA-G mice. Instead, monolayers of adherent cells with macrophage-like appearance developed in cultures from bone marrow of HLA-G mice.

Flow cytometric analyses of GM-CSF/bone marrow cultures revealed distinct phenotypic characteristics of cells from HLA-G mice when stained with antibodies for myeloid markers...
HLA-G mediated immunodeficiency

Fig. 2. Analyses of spleen histology in HLA-G mice. Spleen tissue sections (×40) from CBA (top row) and HLA-G (bottom row) stained with H & E (A and B) and antibodies specific for CD11c (C and D) and CD11b (E and F). Photomicrographs (×200) of primary bone marrow cultured for 10 days without added growth factors (G and H).

Fig. 3. Flow cytometric analyses of splenocytes isolated from HLA-G mice. Splenocytes were stained with T cell (CD3, CD4, CD8), B cell (B220), and myeloid (F4/80) and pan-NK cell (DX5) markers, and analyzed by two-color flow cytometry. Different gates were set for lymphoid (FSC, SSC) or myeloid/NK (FSC, SSC) marker analysis. Figures indicate percentage of total gated cells falling into selected quadrants. Data is representative of multiple analyses.

(CD11b, CD11c and F4/80) and MHC II (H-2A^k) (Fig. 5). The majority (>90%) of cells derived from culture in GM-CSF were CD11c^+, whether they originated from HLA-G or CBA mice. However, the majority of cells derived from HLA-G bone marrow stained strongly with macrophage-lineage markers CD11b and F4/80, unlike cells elicited from CBA bone marrow. In addition, cells spontaneously expressing high levels of MHC II, which constituted 20% of cells derived from CBA mice, were absent in cultures derived from HLA-G mice. Additional analyses revealed that most cells cultured in GM-CSF expressed the co-stimulatory marker CD86 and stained weakly for CD80 (Fig. 6). However, populations of cells expressing high levels of CD86 were absent in cultures from HLA-G bone marrow (Fig. 6); these CD86^hi cells also expressed MHC II at high levels (data not shown). These data showed that subsets of mature differentiated myeloid...
Fig. 4. T cell proliferative responses (A and B) and NK cell cytolytic activity (C). Responder T cells isolated from HLA-G (●) and CBA (○) lymph node were co-cultured with irradiated stimulators prepared from BALB/c and C57BL/6 mice. T cell proliferation was assessed by incorporation of [3H]thymidine as described in Methods. NK cytolytic activity of purified NK cells was assessed on NK sensitive YAC-1 targets (C). Data is representative of at least three separate experiments.

cells expressing high levels of MHC II and CD86 did not develop when bone marrow from HLA-G mice was cultured with GM-CSF. Thus, spontaneous maturation of myeloid DC, which display these phenotypic characteristics, appeared to be compromised in HLA-G mice. To examine whether the apparent defect in DC maturation could be overcome, GM-CSF/bone marrow cultures from HLA-G mice were supplemented with LPS to deliver more potent activation to myeloid APC precursors (Fig. 6). MHC II and CD86 staining profiles were identical when bone marrow from HLA-G and CBA mice was cultured with GM-CSF and LPS. From these data we conclude that immature DC precursors were present in GM-CSF/bone marrow cultures but they did not develop spontaneously into mature DC unless LPS was added to cultures.

To examine whether functional APC developed from HLA-G mice, cells were irradiated and co-cultured with responder cells from allogeneic BALB/c mice and T cell proliferation...
was assessed (Fig. 7). Proliferative T cell responses were decreased significantly when splenocytes from HLA-G mice were used as APC (Fig. 7A). Moreover, cultured bone marrow cells from HLA-G mice failed to elicit proliferative T cell responses when co-cultured with responder T cells from BALB/c (Fig. 7B) and B6 (data not shown) mice. As expected, cells from CBA mice elicited potent T cell responses. Addition of LPS to GM-CSF/bone marrow cultured from HLA-G mice yielded cells that stimulated T cell proliferation, albeit responses were still less than controls (Fig. 7C). These data show that HLA-G mice are deficient in producing APC capable of stimulating T cell proliferation. Moreover, DC cultured from HLA-G bone marrow displayed no APC function at all, suggesting that co-culturing with T cells in vitro did not lead to functional maturation of APC.

**Murine myelomonocytic cells exhibit HLA-G binding activity**

To identify murine cells that might interact with HLA-G molecules we prepared HLA-G tetrameric reagents by refolding HLA-G heavy chains with β2-microglobulin and a synthetic HLA-G-binding peptide. The peptide sequence selected is present in a region of histone H1 conserved in mice and humans. This reagent was used to screen murine splenocytes ex vivo and cultured bone marrow cells for HLA-G binding activity (Figs 8 and 9). HLA-G tetramer binding activity was detected on most CD11c+ cells isolated from CBA spleen

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**Fig. 6.** Single-color flow cytometric analyses of GM-CSF-derived bone marrow cultures with (lower panels) and without (upper panels) activation with LPS (1 µg/ml added on day 6 for 48 h). Staining profiles for H-2E^k^, CD80 and CD86 are shown for cultures originating from HLA-G (thick line) and CBA/ml (thin line) bone marrow. Staining by isotype-matched antibodies is indicated by dotted lines.

**Fig. 7.** Functional analyses of APC from HLA-G mice. (A) Splenocytes from CBA and HLA-G mice were irradiated and used to stimulate T cell proliferative responses as described in Methods. Allogenic responder T cells were prepared from BALB/c. (B) Bone marrow-derived APC were cultured for 8 days with GM-CSF, irradiated and used to stimulate T cell proliferative responses. Allogenic responder T cells were prepared from BALB/c. Bone marrow-derived APC were prepared from CBA and HLA-G mice. (C) GM-CSF-derived bone marrow APC cultured for 48 h prior to use with LPS (1 µg/ml). Data is representative of at least three separate experiments.
after treatment with collagenase, which releases stromal cells. Higher proportions of CD11c⁺ splenocytes from HLA-G mice exhibited HLA-G tetramer binding activity and mean staining intensities were elevated on these cells. HLA-G tetramer binding activity showed similar patterns on CD11c⁺ cells elicited from bone marrow cultured with GM-CSF. CD11c⁺ cells bound to HLA-G tetramer and staining intensities were higher on cells derived from HLA-G mice. No binding activity was observed with irrelevant H-2Db tetramer (Fig. 8). These data revealed that many murine CD11c⁺ myeloid cells in vivo and in vitro expressed receptors potentially able to recognize and confer responsiveness to human HLA-G molecules.

Discussion
The studies described in this report were conducted to investigate the unexpected observation that cellular immune responses were defective in transgenic mice expressing human HLA-G molecules. Our findings show that expression of HLA-G in mice resulted in inhibition, but not complete extinction of T cell responsiveness directed against allogeneic skin grafts. The most likely explanation for this phenomenon is failure to mobilize the afferent arm of the immune response efficiently since in vitro T cell responsiveness is normal, while development of mature CD11c⁺ DC with T cell APC functions from cultured bone marrow is defective. Therefore, we conclude that maturation of functional myeloid APC is compromised in HLA-G mice.

T cells are necessary and sufficient for allograft rejection (23). Cytolytic NK cells can also contribute to tissue rejection in some circumstances (24,25). The prolonged periods required to reject MHC and minor histocompatibility complex mismatched skin allografts were striking demonstrations of the suppressed state of cellular immune responses in HLA-G mice. Fully immunocompetent mice reject MHC-disparate skin allografts rapidly and grafts are normally shed by the latter half of the second week following initial engraftment. Grafts applied to HLA-G mice were not only rejected more slowly but their appearance during rejection was indicative of altered cellular immune processes. Grafts applied to HLA-G mice slowly withered away and had a 'ghosted' appearance characteristic of indirect allorecognition of processed graft alloantigens (26–28). In contrast, grafts targeted by direct T cell allorecognition processes rapidly become necrotic and are shed soon afterwards. It is unclear whether defective afferent responses alter development of direct and indirect alloresponses differentially in HLA-G mice.

Our findings strongly suggest that the immunological defect in HLA-G mice resides in the inability to generate effective afferent responses involving APC activation, maturation or migration (29). Dramatic evidence that growth and development of myeloid cells was abnormal was obtained when cells were cultured from bone marrow and spleen. Normally, cellular outgrowths are not observed from these tissues unless growth factors are included in culture medium but cultures from HLA-G mice gave rise to spontaneous outgrowths of CD11b⁺ myeloid cells in the absence of exogenous growth factors. However, GM-CSF-derived bone marrow did not stimulate proliferation of allogeneic T cells. Culture in GM-CSF is widely used to generate mature myeloid DC with potent APC functions from mice. Thus, failure to generate functionally competent APC using this technique suggests that maturation of immunostimulatory DC is defective in HLA-G mice. We know that precursors of mature DC were present in GM-CSF-
derived bone marrow cultures since LPS treatment, which delivers potent DC maturation signals at the doses used in this study, was able to bypass the defect (29). These findings suggest that there is a defect in spontaneous maturation of DC from HLA-G mice. Furthermore, this defect cannot be overcome by signals from activated T cells, which normally provide maturation signals to DC (30). Thus, expression of HLA-G in mice did not prevent development of myeloid precursors capable of differentiating into mature DC APC. Rather, HLA-G expression in mice interfered with processes that normally provoke DC maturation.

Previous studies have focussed on cellular immunity in transgenic mice expressing human MHC I molecules. There are no reports that cellular immunity was suppressed in mice as a consequence of HLA-A, -B, -C or -E expression (31–33). In contrast, suppressed cellular immune responses manifested in all HLA-G mice from two independently bred lineages. This implies that expression of HLA-G caused the immunosuppressed phenotype in HLA-G mice and that this is a unique characteristic of HLA-G molecules. Data presented in this report reveal that most myeloid CD11c+/CD11b− cells in murine bone marrow and spleen express receptors that can recognize HLA-G–peptide complexes. We hypothesize that HLA-G molecules interact with these undefined receptors expressed on myelomonocytic precursors so that maturation of functionally competent APC is compromised. These molecular and cellular interactions may mimic physiologic processes that can interfere with DC maturation in mice, which involve as yet undefined murine HLA-G homologues. Furthermore, analogous processes mediated by human trophoblast cells expressing HLA-G may help to suppress maternal T cell responses to paternally inherited alloantigens.

Recently, it has been reported that HLA-G tetramer binding activity is associated with human myelomonocytic cells rather than NK cells and inhibitory Ig-like receptor molecules, ILT4, may mediate HLA-G binding to blood myelomonocytic cells in humans (34). Based on these findings Allan et al. suggested that the primary role of HLA-G may be the modulation of myelomonocytic cell behavior in pregnancy. Based on findings we report here, we suggest that expression of HLA-G in mice inhibits myelomonocytic cell maturation into functionally competent APC. The molecular mechanisms and cellular processes by which murine cells expressing HLA-G interfere with maturation of DC APC are currently under investigation.

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Abbreviations

- APC: antigen-presenting cell
- B6: C57BL/6
- DC: dendritic cells
- GM-CSF: granulocyte macrophage colony stimulating factor
- HRP: horseradish peroxidase
- ILT: Ig-like transcript
- LCMV: lymphocytic choriomeningitis virus
- LPS: lipopolysaccharide

References


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