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IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes

Philippe Moreau, Francisco Adrian-Cabestre, Catherine Menier, Virginie Guiard, Luc Gourand[1], Jean Dausset[2], Edgardo D. Carosella and **Pascale Paul**

CEA, Service de Recherches en Hémato-immunologie, DSV/DRM, Hôpital Saint-Louis, Centre Hayem, 1 avenue Claude Vellefaux, 75010 Paris, France
[1]Hopital des Bluets, 9 rue des Bluets 75011 Paris, France
[2]Fondation Jean Dausset, CEPH, 27 rue Juliette-Dodu, 75010 Paris, France

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Abstract

HLA-G plays an essential role in feto-maternal tolerance by inhibiting lysis by maternal NK cells. The factors that allow tissue-specific activation of HLA-G gene expression in trophoblasts remain to be characterized. We investigated the potential effect of IL-10, a cytokine which is secreted in placenta, on HLA-G gene transcription in trophoblasts. Using Northern blot, RNase protection assay and RT-PCR analysis, we demonstrated that IL-10 enhances steady-state levels of HLA-G transcription in cultured trophoblast cells. We further tested the effect of IL-10 on HLA-G gene transcription and protein expression in peripheral blood monocytes, showing that IL-10 can up-regulate HLA-G cell surface expression in this cell type. This effect of IL-10 is selective, since classical MHC class I products and MHC class II are down-regulated in monocytes following IL-10 treatment. Induction of HLA-G expression by IL-10 on monocytes may thus play a role in down-regulation of the immune response. We propose that IL-10 secretion by trophoblasts during pregnancy may also influence the HLA class I expression pattern at the feto-maternal barrier, thus protecting the fetus from rejection. This should be taken into consideration in the design of treatment for pathologies of pregnancy.

Introduction

During human pregnancy, the pattern of MHC protein expression in trophoblasts is characterized by the absence of classical MHC class I and class II antigen expression, thereby preventing fetal cell lysis by maternal alloreactive T cells. Instead, non-classical class I HLA-G proteins, which afford protection from maternal NK cells, are specifically expressed in cytotrophoblasts (1–3). In contrast to classical class I products, HLA-G protein exhibits low polymorphism (1). Furthermore, four putative membrane-bound and two soluble protein isoforms of the HLA-G molecule are generated by alternative splicing of the primary HLA-G transcript (1,4). HLA-G is a ligand for killing inhibitory receptors (p49, ILT-2/LIR-1 and ILT-4) found on NK, T cells and myelomonocytic cells, and is thereby thought to play a major role in down-regulation of the immune response (5–11).

HLA-G gene transcription and protein expression are strongly activated in trophoblast cells, and have recently been reported in thymus and endothelial cells (12,13). HLA-G transcriptional activity is basal or null in most other cell types (14,15). The factors responsible for the specific activation of HLA-G in trophoblast cells remain to be defined and could include immunomodulatory factors locally secreted by the placenta during pregnancy. The human placenta secretes steroid hormones and a variety of cytokines, including CSF-1, macrophage colony stimulating factor, IL-1β, IL-6 and transforming growth factor-β (17–23), as well as IL-10, which is produced during all stages of gestation (21,24). In this work we investigate the possible modulatory effect of the immunosupressive cytokine IL-10 on HLA-G gene transcription and protein expression.

IL-10 is also secreted by a variety of other cells, including activated T and B lymphocytes, monocytes, and UV-irradiated keratinocytes (25–28), exhibiting a broad spectrum of biological activities, including anti-inflammatory and immunosuppressive effects (29). While IL-10 inhibits alloantigen-specific proliferative responses, macrophage-dependent antigen presentation and $CD4^+$ T cell proliferation, it also has both inhibitory and stimulatory effects on human

Correspondence to: P. Paul

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$CD8^+$ T cells, according to their state of activation (30), and has a stimulatory effect on B cells and mast cells (31,32).

The synthesis of several T_h1-type cytokines, such as IFN-γ, tumor necrosis factor (TNF)-α and IL-2, in T cells is down-regulated by IL-10 (25). IL-10 has also been shown to inhibit the synthesis of pro-inflammatory cytokines, such as IL-1, IL-6, TNF-β and antigen presentation in activated macrophages (33,34). It promotes the development of a type 2 cytokine pattern by inhibiting IFN-γ production, in T cells and NK cells, by suppressing monocyte/macrophage and dendritic cell IL-12 production (35).

Expression of HLA class I, class II and ICAM-1 molecules is down regulated by IL-10 in IL-10 receptor-positive melanoma cells (36–38) and cytotrophoblast-produced IL-10 has been shown to suppress IFN-γ production in allogenic mixed lymphocyte reactions (24). Taken together these observations suggest that IL-10 may play a key role in protecting the semi-allogenic human fetus from maternal immune responses (24). Production of IL-10 by cytotrophoblast cells that also express HLA-G make it an attractive candidate molecule for modulation of pregnancy-specific proteins involved in regulating immune tolerance.

We therefore investigated the potent role of IL-10 in modulating HLA-G gene expression in trophoblast cells. Quantitative analysis of HLA-G transcription in trophoblasts clearly demonstrates a significant increase in the quantity of HLA-G mRNA after *in vitro* exposure to IL-10 treatment. Since IL-10 also down-regulates various monocyte glycoproteins that play a co-stimulatory role in T cell activation, including CD80, CD86, CD58 and CD54, as well as MHC class II antigens in monocytes (39–42), we further investigated whether IL-10 could also play a role in regulating HLA-G expression in human monocytes. We show here that IL-10 can selectively enhance HLA-G cell-surface expression in human monocytes, while down-regulating classical HLA class I antigens.

Methods

Tissues, cultured cells and induction experiments

Four trophoblasts were obtained from voluntarily interrupted normal pregnancies at 5–7 weeks of gestation (local ethics committee approval was obtained) and immediately dissected into small pieces. Trophoblast explants were incubated at 37°C under an atmosphere of 5% CO_2 in six-well cell culture plates containing Dulbecco's medium without phenol red (Life Technologies, Cergy Pontoise, France) supplemented with 0.1% gentamycin, 1×anti-PPLO agent (Life Technologies), and 10% FCS purified through dextran-coated charcoal (Sigma, St-Quentin-Fallavier, France). Cells either were or were not incubated with 50 ng/ml recombinant human IL-10 (sp. act. 5×10^5 U/mg; PromoCell, Heidelberg, Germany) for 72 h.

Peripheral blood monocytes were isolated from 10 volunteer donors as follows: mononuclear cells were separated from blood cells by Ficoll-Hypaque gradient 1077. Monocytes separation was performed using two methods. (i) Mononuclear cells were depleted of B cells by immunoabsorption on anti-CD19-coated Dynadeads (Dynal, Oslo, Norway). Monocytes were enriched by 1 h adherence to tissue culture flasks at 37°C in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated fetal bovine serum, fungizone and gentamycin. Non-adherent cells were removed and adherent monocytes placed in fresh culture medium. (ii) Alternatively, monocytes were labeled with super-paramagnetic MicroBeads conjugated with monoclonal mouse anti-human CD14 antibodies (Tebu, Le Perray-en-Yvelines, France). Positive separation from mononuclear cells was performed on a LS^+/VS^+ column in a magnetic cell separator VarioMACS (Miltenyi Biotec, Bergish Gladbach, Germany). Monocytes were treated for 48 h with IL-10 at a concentration of 25 or 200 U/ml (PharMingen, Le Pont de Claix, France).

Radioactive labeling of riboprobes

Single-stranded radiolabeled RNA probes were synthesized using the MAXIscript *in vitro* transcription kit (Ambion, Austin, TX) in the presence of T7 RNA polymerase and 5 μl [α-^{32}P]CTP (Amersham, Les Ulis, France) according to the manufacturer's procedure. Cyclophilin standard template was obtained from Ambion. The HLA-G template was obtained by PCR amplification of an HLA-G genomic fragment covering a part of the 3′-untranslated (UT) region. The forward primer was G.1089F (5′-CCCTTTGTGACTTCAAGAAC) and the reverse primer, which included a 29 base region used to generate a T7 promoter during PCR (underlined), was T7G.1250R (5′-GGATCCTAATACGACTCACTATAGGGAGG-TTATAGCTCAGTGGCCCAC). The probes were gel purified by 8 M urea–6% acrylamide gel electrophoresis. The gel was then exposed to X-ray film. The area of the gel containing the labeled full-length transcript was submerged in 350 μl of probe elution buffer provided with the Ambion kit.

Northern blot

Total RNA from trophoblast cultures was prepared using RNA NOW reagent (Biogentex, Seabrook, TX) according to the manufacturer's recommendations. RNA (15 μg) was checked by electrophoresis in a 1.5% agarose denaturing gel (18% formaldehyde, 100 mM sodium acetate, 20 mM EDTA). RNA was transferred to a nylon membrane (Schleicher & Schuell, Ecquevilly, France) and UV-linked. The membrane was hybridized with ^{32}P-labeled HLA-G riboprobe obtained as described above and rehybrized following dehybridization with radiolabeled GAPDH probe prepared as described (43). The membrane was exposed to a molecular imager (BioRad, Ivry-sur-Seine, France) for quantification of HLA-G transcriptional activity. The values obtained for HLA-G signals were normalized to the constitutively expressed GAPDH signal.

RNase protection assays

Protection of HLA-G transcripts was carried out using a HybSpeed RPA kit (Ambion) according to the instruction manual. Briefly, 5 μg of total RNA isolated as described above, 5×10^5 c.p.m. of HLA-G riboprobe and 5×10^5 c.p.m. of cyclophilin riboprobe were hydridized for 10 min in 10 μl of Hybspeed hybridization buffer. RNase A/T1 mix at a final concentration of 1/100 was then added for 30 min. Radiolabeled protected fragments were precipitated and separated on 5% acrylamide:bis acrylamide (19:1) native gel

under 100 V. The gel was dried and exposed to a molecular imager (BioRad) for quantification.

RT-PCR analysis

Complementary DNAs were prepared from 5 μg of total RNA, using oligo(dT)$_{12–18}$ priming and M-MLV reverse transcriptase (Life Technologies) at 42°C for 1 h. G.257 (exon 2-specific) and G.1225 (3′-UT region) pan-HLA-G primers were used to amplify all alternative forms of HLA-G transcripts for 35 cycles, as previously described (44). Specific amplification of HLA-G transcripts corresponding to the various isoforms was carried out with the following primer sets: G.526 (exon 3) and G.1225 (3′-UT) for G1 (full-length) and G4 (minus exon 4), G.526 (exon 3) and G.i4b (intron 4) for G5 (full length with intron 4), G.-3 (overlapping exons 2 and 4) and G.1216 (3′-UT) for G2 (minus exon 3) and G6 (minus exon 3 with intron 4), and G.-3–4 (overlapping exons 2 and 5) and G.1216 (3′-UT) for G3 (minus exons 3 and 4). Classical HLA-class I cDNA were amplified using HLA-5P2 (5′-UT) and HLA-3PI (3′-UT) primer sets, as described (45). Co-amplification with β-actin primers (Clontech, Heidelberg, Germany) was carried out during the last 16 amplification cycles. PCR products were analyzed by Southern blot, as previously described (45). The following HLA-G-specific probes were used: G.I4F (intron 4-specific) and G.1200 (3′-UT). The probes used to detect β-actin and classical HLA-class I cDNA have been previously described (45).

mAb and flow cytometry

The following mAb were used: anti-CD14 (UCHM-1 clone, FITC-conjugated mouse IgG2a; Sigma), 87G (anti-HLA-G), kindly provided by Dr D. E. Geraghty (46), 3G11 (anti-HLA-A), kindly provided by Dr L. P. De Waal (47), Tu149 (anti-HLA-B/C, FITC-conjugated mouse IgG2a; Caltag, Burlingame, CA), HLA-DR (anti-HLA-DR, L243 clone, FITC-conjugated mouse IgG2a; Becton Dickinson, Le Pont-de-Claix, France). FITC-conjugated mouse IgG2a was used as the control. 3G11 and 87G were stained using a goat anti-mouse IgG antibody conjugated with phycoerythrin (Immunotech, Marseilles, France). In the corresponding analysis, mouse total IgG and goat F(ab′)$_2$ fragment anti-mouse IgG (H + L) antibody conjugated with phycoerythrin (Immunotech) were used as the control. Double staining experiments were conducted with 87G mAb and phycoerythrin (PE)–(Fab′)$_2$ Goat anti-mouse IgG (H + L) as a secondary reagent followed by FITC-labeled CD14 mAb. To eliminate non-specific binding, FcR were blocked by two procedures: for 30–60 min at 4°C as previously described (48,49) or by incubation 30 min in 5% human AB serum and 10 μl/ml normal rabbit serum (Dako, St-Quentin-en-Yvelines, France). Cells were analyzed in a flow cytometer (FACS Vantage; Becton Dickinson). The following four data parameters were collected in Listmode files: linear forward-scatter, linear side-scatter, log FITC and log PE fluorescence. Off-line analysis was conducted using Lysys II software as supplied by Becton Dickinson. Region R1, representing CD14$^+$ events, was selected to include all monocytes on the basis of forward and side light scatter.

Immunocytochemical staining

CD14$^+$ MACS-purified monocytes were isolated as described above and cultured in Lab-Tek II Chamber Slide system (Nalge Nunc, Naperville, IL) as described above and fixed for 8 min in cold acetone. FcR were blocked by 5% human AB serum and 10 μl/ml normal rabbit serum (Dako). Staining was carried out with Dako EnVision+ system according to instructions supplied in the kit. Briefly, endogenous peroxidase was inhibited by 0.03% hydrogen peroxide containing sodium azide for 5 min. Primary mAb 87G (anti-HLA-G) or IgG2a (control) were applied for 30 min. Incubation with peroxidase-labeled polymer conjugated to goat anti-mouse Ig was performed for 30 min followed by the addition of 3,3′-diaminobenzidine substrate–chromogen solution for 10 min. Nucleus staining was obtained by slide immersion in hematoxylin (Dako) for 4 min.

Results

IL-10 increases HLA-G mRNA levels in trophoblast cells

To analyze the potential of IL-10 to activate HLA-G transcription in trophoblast tissue, we incubated organ explants from the same trophoblast for 72 h in the absence or presence of IL-10. Northern blot analysis and quantification of total RNA from untreated or treated first trimester trophoblasts demonstrated that IL-10 treatment increased the rate of HLA-G mRNA transcription at least 7-fold compared to the quantity of HLA-G mRNA observed in untreated cells (Fig. 1A). RNase protection assays also demonstrated that steady-state levels of HLA-G mRNA were increased 7.2-fold following IL-10 treatment of trophoblast explants (Fig. 1B).

In order to analyze IL-10-induced patterns of HLA-G alternative transcription, we used HLA-G-specific primers that amplify isolated HLA-G mRNA forms. Southern blotting of HLA-G RT-PCR products (Fig. 2) revealed that IL-10 treatment enhanced all alternatively spliced transcripts, including G5 and G6, which encode the soluble HLA-G isoforms.

IL-10 up-regulates HLA-G expression in peripheral blood monocytes

To further investigate the effect of IL-10 on HLA-G gene transcription in other cell types, we focused on peripheral blood monocytes that express classical HLA class I transcripts and proteins and bear IL-10 receptors (50). CD19-depleted adherent cells from three donors were incubated in medium in the presence or absence of IL-10 for 48 h. HLA-G transcripts were analyzed by RT-PCR, using the pan-HLA-G primer and HLA-G5-specific primer sets, which amplify the alternative form that encodes the HLA-G5 soluble protein (Fig. 3A). As previously published (51), we observed that the HLA-G gene was moderately transcribed in monocytes. We also noted that HLA-G transcriptional signals were weakly enhanced following IL-10 exposure (Fig. 3A). We then analyzed HLA-G protein expression in IL-10-treated or untreated monocytes using the 87G anti-HLA-G-specific mAb. Figure 4 shows that the percentage of HLA-G$^+$ cells and the HLA-G cell surface density were increased following IL-10 treatment. Mean values obtained in three experiments were 26.6% HLA-G$^+$ cells in the absence of IL-10 and 51.1% HLA-G$^+$ cells after IL-10 treatment. Mean values for HLA-G cell-surface density were 38.9 for untreated and 50.7 for IL-10-treated cells respectively.

The induction of HLA-G expression at the surface of mono-

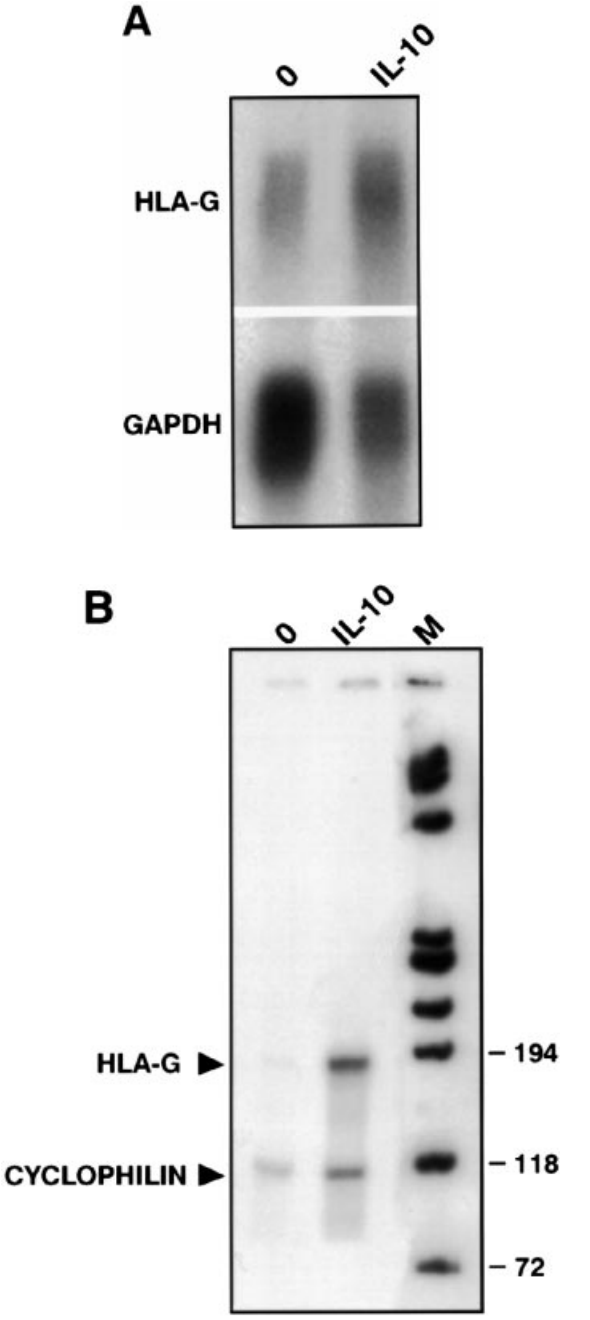

Fig. 1. IL-10 increase in HLA-G mRNA levels in trophoblasts organ explants of first trimester of gestation after 72 h of IL-10 treatment. (A) Northern blot analysis using ^{32}P-labeled antisense RNA corresponding to the 3′-UT region of HLA-G gene. A ^{32}P-labeled GAPDH DNA probe was used in a second hybridization step to quantify RNA. '0' and 'IL-10' indicate RNA obtained from the untreated or IL-10-treated (50 ng/ml) trophoblast respectively. Comparison of HLA-G:GAPDH signals was carried out with a scanning densitometer. (B) RNase protection analysis carried out by the protection of HLA-G and cyclophilin (internal quantification control) transcripts with specific ^{32}P-labeled riboprobes. After digestion by an RNase A/T1 mix, the hybridized products were separated by native PAGE. Comparison of HLA-G:cyclophilin signals was carried out with a scanning densitometer. 'M' indicates a mol. wt marker.

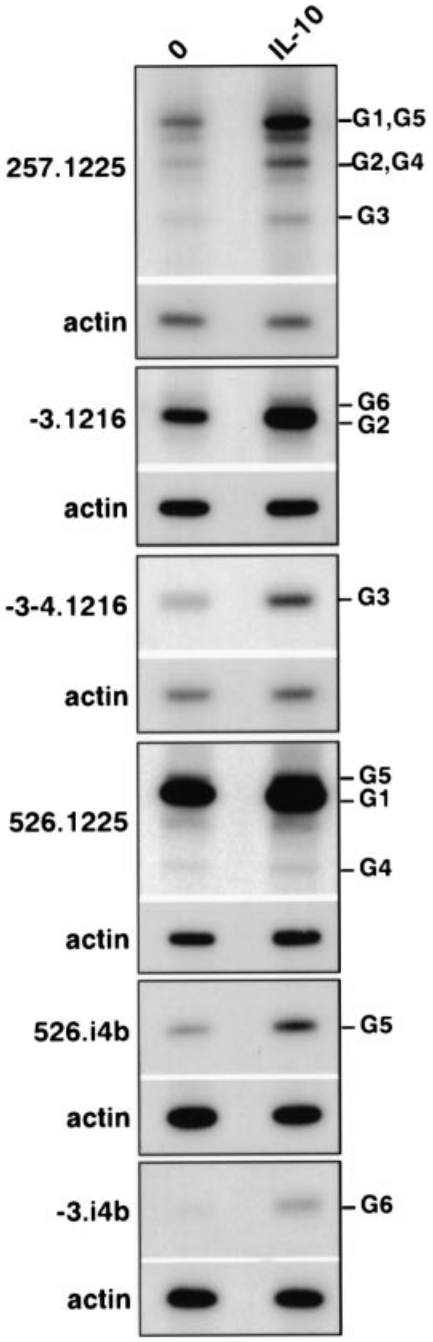

Fig. 2. RT-PCR and Southern blot analysis of alternatively spliced HLA-G mRNA in trophoblast cells after 72 h IL-10 induction. Primer sets used to discriminate HLA-G and β-actin products are indicated on the left. Bands corresponding to HLA-G-specific isoforms were revealed by hybridization with G.1200 probe (3′-UT region), except for bands corresponding to G.526-G.i4b and G.-3-G.i4b PCR products, which were revealed by G.I4F probe (intron 4 specific). Bands G1 (full length transcript), G2 (minus exon 3), G3 (minus exons 3 and 4) and G4 (minus exon 4) correspond to transcripts encoding membrane-bound HLA-G isofoms; G5 (plus intron 4) and G6 (minus exon 3, plus intron 4) correspond to transcripts encoding soluble HLA-G isoforms.

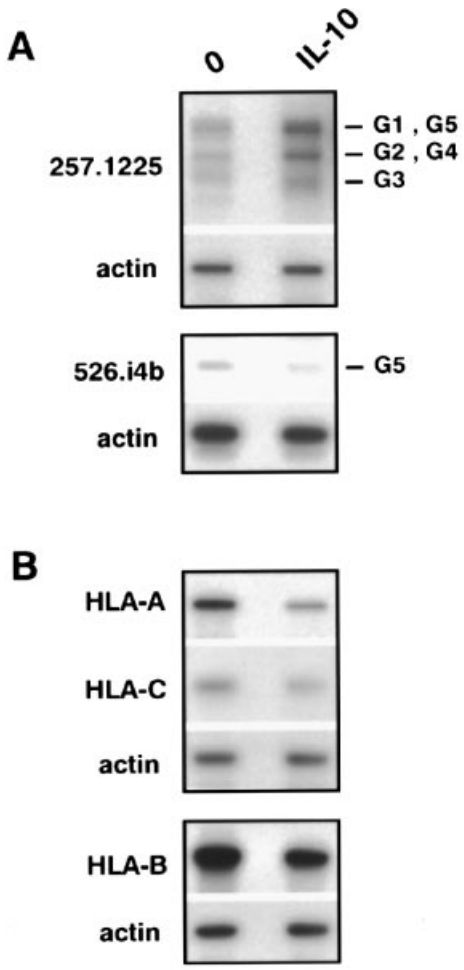

Fig. 3. RT-PCR and Southern blot analysis of HLA-class I transcription in human monocytes cultured without ('0') or with IL-10 ('IL-10') for 48 h. (A) Specific analysis of HLA-G transcripts with pan-HLA-G primers (G.257-G.1225) and primers for specific amplification of G5 encoding the soluble isoform (G.526-G.i4b). PCR products were hybridized with G.1200 (3′-UT) and G.I4F (intron 4) probes respectively. (B) Specific analysis of classical class I transcripts using pan-class I primers. Locus-specific bands were revealed using HLA-A-, -B- and -C-specific probes (see Methods).

cytes following IL-10 treatment was further confirmed by double-staining experiments using 87G and CD14 mAb (Fig. 5). To access the indirect impact of monocyte purification methods on HLA-G activation, monocytes were obtained simultaneously using two purification methods (positive CD14 MACS selection or CD19 depletion and cell adherence). The analysis of seven distinct donors by double staining allowed us to access the heterogeneity of the level of enhancement of HLA-G expression following IL-10 treatment. Among these donors, three revealed a significant increase in HLA-G cell-surface expression after IL-10 treatment and exhibited basal HLA-G expression independently of the method used (Fig. 5); two only exhibited slight increase in HLA-G expression and no detectable effect of IL-10 on HLA-G expression was noted on two donors (not shown).

Further confirmation of HLA-G protein expression in IL-10-treated monocytes was given by immunocytochemistry analysis of treated monocytes using the anti-HLA-G specific 87G mAb (Fig. 6).

IL-10 down-regulates classical HLA class I cell-surface expression of peripheral blood monocytes

The effect of IL-10 on classical HLA class I transcription in monocytes was analyzed by RT-PCR, using pan-class I primer. PCR products were hybridized with HLA-A-, HLA-B- or HLA-C-specific probes. We noted that classical HLA class I transcription was only slightly down-regulated after IL-10 treatment (Fig. 3B). Cell-surface MHC class I expression was further analyzed, using 3G11 (anti-HLA-A) and Tu149 (anti-HLA-B/C) mAb. As shown in Fig. 4, the capacity of IL-10 to recruit cells in the HLA-G$^+$ population was counterbalanced by a concomitant decrease in HLA-A and HLA-B/C cell-surface expression. As previously described (42), down-regulation of MHC class II antigens expression was also observed.

Discussion

IL-10 down-regulates classical HLA class I and class II molecules in melanoma cells and HLA class II molecules in monocytes (34,36,42), suggesting a potential role for this cytokine in the induction of immune tolerance.

HLA-G has recently been described as a ligand for MHC class I-specific killing inhibitory receptors expressed on NK, T and myelomonocytic cells, and thus could be a modulator of immune responses. We therefore investigated the effect of IL-10 on HLA-G expression in human peripheral blood monocytes, which play a crucial role in the immune response to infection and trauma. Our results demonstrate the ability of IL-10 to enhance HLA-G cell-surface expression in these cells. IL-10 enhancement of HLA-G expression on the cell-surface is counterbalanced by its concomitant capacity to down-regulate classical MHC class I and class II antigen cell-surface expression. IL-10 is known to protect target cells from allo-specific cytotoxic T lymphocytes and has been associated with a decrease in HLA class I molecules in melanoma cells (36–38). Moreover, the fact that monocytes express HLA-G protein after IL-10 induction suggests a role for this non-classical MHC antigen in the regulation of inflammatory responses.

Basal levels of HLA-G cell surface expression were observed in some untreated monocytes. This observation has been previously reported (51), but remains controversial, as both basal and IL-10- or IFN-γ-induced HLA-G expression could not be detected by others authors on monocytes purified from three different blood donors (52). HLA-G expression observed in untreated monocytes may be the reflect of *in vivo* expression in this cell type, which may be dependent on the physiological state of the donor tested. Alternatively, one cannot exclude that HLA-G expression in untreated monocytes may result from *in vitro* activation of monocytes induced by the purification procedure (such as phagocytose and adherence) or culture conditions. The physiological state and microenvironment of monocytes may also contribute to their ability to respond to cytokine stimulation. These different aspects could in part explain reported discrepancy of HLA-

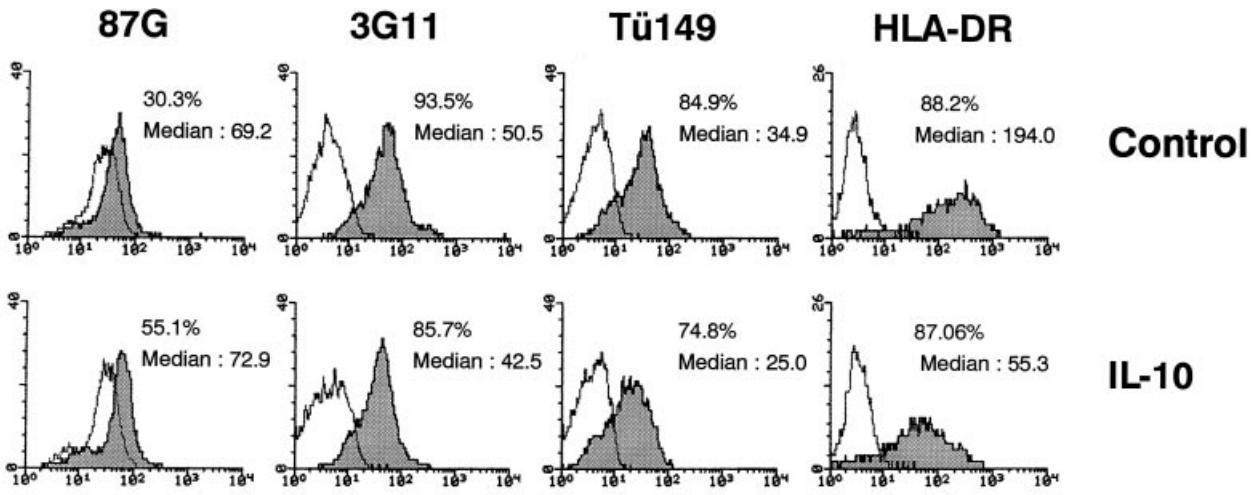

Fig. 4. Flow cytometry analysis of HLA expression in blood monocytes incubated with medium alone (control) or medium containing IL-10. Cells were tested for binding of the following mAb (bold profiles): 87G (HLA-G), 3G11(HLA-A), Tu149 (HLA-B/C) and HLA-DR. Controls were the same cells stained with an isotype-matched control antibody (light profiles).

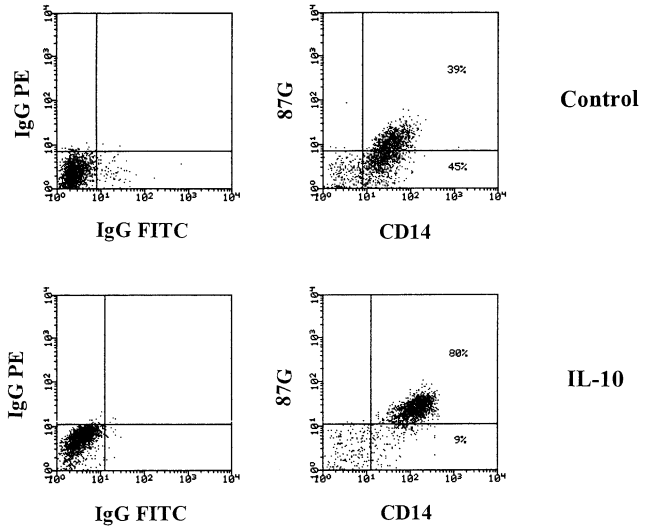

Fig. 5. Effects of IL-10 treatment on HLA-G expression after 48 h culture of monocytes purified by $CD14^+$ selection prior to treatment. Monocytes were double labeled with 87G (HLA-G) and FITC-conjugated CD14 mAb. 'Control' corresponds to monocyte culture in the absence of IL-10.

G expression detected both in untreated and IFN-γ-treated cultured monocytes (51,52). Our data and the reported demonstration of the induction of HLA-G molecules by IFN-γ in the monohystiocytic cell line U937 (53,52) suggest that inflammatory conditions may also be associated with increased expression of this molecule. The baseline status of HLA-G expression and its ability to be up-regulated by both these cytokines could thus play a role in regulating immune responses during inflammatory processes.

IL-10 is produced by human placenta trophoblasts *in vivo* (24). The present study demonstrates that *in vitro* IL-10 treatment induces HLA-G mRNA transcription in trophoblast organ culture. The amount of HLA-G mRNA is significantly increased (~7-fold), compared with untreated cells. In addition, RT-PCR analysis demonstrates an increase in the level of all HLA-G mRNA transcript isoforms, including those that encode soluble proteins in IL-10-treated trophoblast culture.

IL-10 is therefore a candidate molecule for a strong stimulatory signal for HLA-G antigen expression in trophoblast cells. In agreement with this hypothesis, there is now evidence for a role of IL-10 in pregnancy success (54,55). In particular, reduced IL-10 production has been reported in pathological pregnancies, distinguishing them from normal ones (55).

In view of published data (55–57), IL-10 production localized in placenta might have immunosuppressive activities, as observed for monocytes. Moreover, in the light of the effect of IL-10 on monocyte HLA protein expression, we can postulate that placental IL-10 could have a dichotomous effect on HLA expression, inducing HLA-G expression, while down-regulating classical class I and class II antigens. Modulation of MHC class I expression by IL-10 may result from molecular mechanisms which include transcriptional regulation of HLA-G expression in trophoblasts and post-transcriptional regulation in monocytes, since enhancement of HLA-G transcript expression in monocytes was low after IL-10 treatment. We also failed to observe significant down-regulation of classical HLA class I gene transcription in monocytes, suggesting a possible effect of IL-10 on post-transcriptional mechanisms. As previously observed in murine tumor cell lines expressing IL-10 (58) or B cells exposed to Epstein–Barr virus-encoded IL-10 (59), IL-10 may down-regulate classical class I expression through TAP proteins. It has recently been suggested that the constitutive absence of classical HLA class I cell-surface expression in term villous cytotrophoblasts and syncytiotrophoblasts is also likely to be due to the lack of transporter protein (60).

In conclusion, our work demonstrates that IL-10 has a dual effect on MHC class I and class II expression, and could thus act as a major modulator of immune tolerance. It is able both to enhance HLA-G cell-surface expression and to down-regulate classical HLA class I and class II antigens on

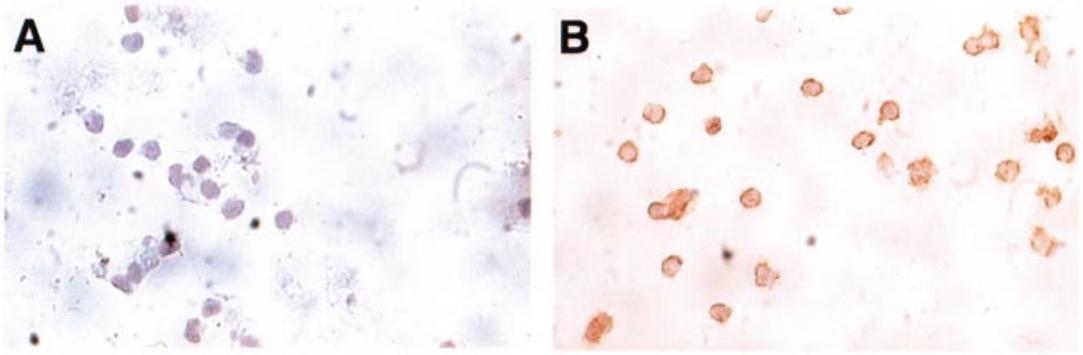

Fig. 6. Immunocytochemistry detection of HLA-G protein expression in monocytes stimulated with IL-10 (25U/ml). (A) Control experiment with IgG2a mAb. (B) Staining with 87G mAb.

the cell surface of monocytes, and thus might be able to regulate NK and T lymphocyte responses. These characteristics suggest the potential clinical use of IL-10 as a potent immunosuppressor in transplantation and autoimmune diseases.

Previous results have correlated IL-10 expression in patients with stable liver graft (61). IL-10 production has also been associated with the establishment of tolerance in SCID patients transplanted with HLA-mismatched hematopoietic stem cells (62). Other effects of *in vivo* IL-10-treatment have already been demonstrated: i.v. IL-10 injection is able to inhibit pro-inflammatory cytokine production and IL-10 therapy has beneficial effects in patients with Crohn's disease (63) or psoriasis (64), in both of which IL-10 deficiency has been described.

The lack of HLA-G expression in trophoblasts could also be implicated in the recurrent spontaneous abortions or poor outcome of pregnancy associated with malaria, in which reduced IL-10 levels are observed (65). Altered expression of HLA-G on extravillous trophoblasts has also been described in pre-eclampsia (66). The clinical use of IL-10 could therefore also be considered in pathologies of pregnancy.

We recently demonstrated that expression of the non-classical HLA-G gene in melanoma cell lines enables these cells to inhibit NK lysis (45,67). IL-10 production by melanoma cells has also been described (38). In view of our previous results, it is possible that IL-10 may contribute to the activation of HLA-G expression in melanoma cells, thereby participating in the escape of tumors from immunosurveillance.

The dual effects of IL-10 on MHC class I molecule expression should be taken into consideration for possible clinical use of IL-10 in the treatment of autoimmune disease and in organ transplantation.

Acknowledgements

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Abbreviations

PE	phycoerythrin
UT	untranslated
TNF	tumor necrosis factor

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