COMMENTARY

HLA-G5 expression by trophoblast cells: the facts

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The non-classical HLA Class I molecule HLA-G was discovered at the fetal–maternal interface, but over the past few years, the tissue distribution of HLA-G in normal tissues has been found to be broader than originally reported: (i) HLA-G molecules have been detected from oocyte to blastocyst stage, then in invasive trophoblast, amniotic fluid, endothelial cells from the chorionic villi and erythroid cells in all organs sustaining primitive to definitive erythropoiesis and (ii) in adult tissues, HLA-G antigens have been detected in thymic epithelial cells, in the epithelium, endothelium and keratocytes from cornea and in cells of the erythropoietic lineage from bone marrow. Additionally, HLA-G ectopic expression was demonstrated in various pathological situations such as cancer, transplantation, viral infection, inflammation and auto-immune diseases (reviewed in Carosella et al., 2003). Concerning soluble HLA-G, both shed HLA-G1 and HLA-G5 proteins have been detected in various body fluids, such as amniotic fluid and serum from pregnant women (Hamai et al., 1999; Puppo et al., 1999; Rebmann et al., 1999), cancer patients (Adrian Cabestre et al., 1999; Ugurel et al., 2001) and transplanted patients (Lila et al., 2000, 2002).

The function of the non-classical HLA Class I molecule HLA-G was initially described in the context of fetal–maternal tolerance where HLA-G expression by classical HLA Class I-negative cytotrophoblast protects this fetal tissue against destruction by natural killer (NK) cells of the mother (Rouas-Freiss et al., 1997a,b). Since then, the capability of HLA-G to inhibit immune responses has been broadened to inhibition of peripheral NK cells and CTLs (Rouas-Freiss and Kirszenbaum et al., 1997; Riteau et al., 2001), inhibition of allogeneic responses (LeMaoult et al., 2004; Mitudoerffer et al., 2005), induction of regulatory cells (LeMaoult et al., 2004; Ristich et al., 2005), up-regulation of inhibitory receptor expression (LeMaoult et al., 2005) and inhibition of dendritic cell maturation (Ristich et al., 2005). This has positioned HLA-G as a molecule capable of significantly contributing to tolerance of allografts (Lila et al., 2002; Creput et al., 2003) and immune escape of tumours (Paul et al., 1998; Wiendl et al., 2002; Bukur et al., 2003a,b; Singer et al., 2003; Nuckel et al., 2005; Rouas-Freiss et al., 2005) and virus-infected cells (Onno et al., 2000; Lozano et al., 2002; Barel et al., 2003; Panguil et al., 2004). These immunosuppressive properties are shared by the HLA-G1 and the secreted HLA-G5 proteins. In addition, the soluble HLA-G isoforms (i.e. both shed HLA-G1 and HLA-G5) can induce apoptosis of activated CD8+ T and NK cells through ligation with CD8 (Carosella et al., 2003).

In a recent article (this issue of Mol Hum Reprod), Blaschitz et al. have re-investigated what was the nature of the soluble HLA-G molecules that were expressed by human trophoblasts and claim that the only soluble form of HLA-G that is produced by trophoblast is the shed HLA-G1 and not the secreted isoform, HLA-G5.

There are several structures of soluble HLA-G proteins: (i) shed, i.e. derived from the release of membrane bound HLA-G isoforms, such as HLA-G1 (HLA-G1s for HLA-G1 shedding) and (ii) secreted, i.e. directly expressed as soluble isoforms, such as HLA-G5. Indeed, the HLA-G primary transcript is alternatively spliced and can lead to the generation of seven alternative mRNAs that encode four membrane-bound (HLA-G1, G2, G3 and G4) and three secreted (HLA-G5, -G6 and -G7) protein isoforms. The secreted HLA-G isoforms are devoid of transmembrane and cytoplasmic parts because of the presence of a stop codon in intron 4 (HLA-G5 and -G6) or intron 2 (-G7) leading to a C-terminal tail specific for these soluble forms. The secreted full-length HLA-G5 isoform is a 37 kDa glycoprotein that retains a1, a2 and a3 domains, but includes an intron 4 sequence, yielding a specific open reading frame that encodes 21 amino acids linked to the a3 domain and excludes the transmembrane domain (Lee et al., 1995). It is against this intron-encoded sequence that all anti-HLA-G5 specific mAbs were raised. Thus, due to the availability of these antibodies, such as the monoclonal antibody 16G1 (Lee et al., 1995), the polyclonal PAG5-6 antibody (Paul et al., 2000), and the recently described 5A6G7 (Le Rond et al., 2004), the expression of secreted isoforms was investigated. Other antibodies specific for the extracellular domain of membrane-bound HLA-G1 do not discriminate between HLA-G1 and HLA-G5 (Table I).

Blaschitz et al. in their article, this issue, have revisited the expression of HLA-G by fetal tissues. The expression of HLA-G, including soluble HLA-G proteins by placental cells has been the centre of investigations for years, ever since monoclonal antibodies allowing discrimination between membrane HLA-G1 and soluble HLA-G5/G6 isoforms were made available. In this regard, the group of D.Geraghty was in 1995 the first to produce and describe mAbs, including 16G1, reacting specifically with the intron-4 containing HLA-G5/G6 proteins (Lee et al., 1995). In 1997, the very same author, who publishes today that there is no HLA-G5 in trophoblast using the 16G1 mAb, tested the 16G1 mAb on serial cryosections of first-trimester placenta and published that this mAb stains the highly proliferative extravillous cytotrophoblast which is also known to highly express membrane bound HLA-G1 (Blaschitz et al., 1997). Obviously, past and present results from this group conflict. It is intriguing that this was not explained in their current manuscript. In our opinion, the authors might seriously consider that their present batch of 16G1 is no longer a reliable tool. In this regard, during the first international workshop on HLA-G (Paris, 2000), we have warned the scientific
Despite this inter-batch liability, the group of J. Hunt in 1998 as well as that of D. Geraghty in 2003 have clearly shown that soluble HLA-G5/-G6 proteins were expressed by trophoblast cells (Chu et al., 1998; Ishitani et al., 2003). Notably, they verified their results by blocking the staining with 16G1 mAb by the addition of the 20-mer synthetic peptide used to generate this mAb (Ishitani et al., 2003). This is more than anyone else has done to prove that the obtained results were not artefactual. Finally, in 2002, our group produced a novel anti-HLA-G5/-G6 mAb called 5A6G7. We first validated this mAb on transfected cells (Le Rond et al., 2004) and then on first-trimester trophoblast sections showing staining in both trophoblast cells, erythroid precursors and endothelial cells, as shown in Figure 1 (Menier et al., 2004). The 5A6G7 mAb is now commercially available and validated by Exbio by checking its reactivity on trophoblast sections and making sure it stains extravillous cytotrophoblast cells. A validation figure obtained by Exbio for the currently marketed batch of 5A6G7 is shown in Figure 1 Exbio. Our own analyses with the commercial as well as our home-produced 5A6G7 mAb show that in addition to extravillous cytotrophoblasts, erythroid cells are stained, as previously reported (Menier et al., 2004) (Figure 2). We also enclosed two figures where the 4H84 (Pan HLA-G proteins) and 5A6G7 (anti-HLA-G5/G6) mAbs were tested under similar conditions on serial sections of first-trimester trophoblast. Results clearly show that both mAbs stained extravillous trophoblast with distinct intensity, the highest being observed with 4H84.

Our main criticism about this article is that since Blaschitz et al. contradict the entire literature on the topic of HLA-G5 expression by trophoblasts, by using methodologies which have been used before and gave opposite results in other hands, it is our belief that rather than HLA-G5 expression by fetal tissues, it is their technical approaches that should be revisited. In this regard, several technical workshops have been organized in 2000, 2003 and 2004 to validate tools and protocols for HLA-G analysis.

Finally, from a diagnostic point of view, it seems to us that distinguishing between HLA-G5 and shed HLA-G1 matters not. Indeed, it is the presence or absence of soluble and/or secreted HLA-G that is important, to distinguish between normal and pathological situation

Table I. Antibodies commonly used for HLA-G detection

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>Availability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEM-G/09</td>
<td>Non-denatured HLA-G1+G5</td>
<td>Exbio, Praha</td>
<td>Menier et al. (2003)</td>
</tr>
<tr>
<td>87G</td>
<td>Non-denatured HLA-G1+G5</td>
<td>Exbio, Praha</td>
<td>Lee et al. (1995)</td>
</tr>
<tr>
<td>4H84</td>
<td>Denatured HLA-G1 domain (HLA-G1–G7)</td>
<td>M. McMaster</td>
<td>McMaster et al. (1995)</td>
</tr>
<tr>
<td>MEM-G/01</td>
<td>Denatured HLA-G1 domain (HLA-G1–G7)</td>
<td>Exbio, Praha</td>
<td>Menier et al. (2003)</td>
</tr>
<tr>
<td>MEM-G/02</td>
<td>Denatured HLA-G1 domain (HLA-G1–G7)</td>
<td>Exbio, Praha</td>
<td>Menier et al. (2003)</td>
</tr>
<tr>
<td>16G1</td>
<td>Denatured and non-denatured HLA-G5/G6</td>
<td>D. Geraghty</td>
<td>Lee et al. (1995)</td>
</tr>
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Figure 1. HLA-G5 is expressed by extravillous cytotrophoblast. Paraffin-embedded sections of first-trimester trophoblast tissue were stained with commercial anti-HLA-G5 5A6G7 mAb by Exbio, Praha, as a validation test for 5A6G7 mAb. Pan-HLA-G MEM-G/02 was used as control. Red staining indicates positivity. Only extravillous cytotrophoblast, i.e. cell island trophoblast is positive.

Figure 2. HLA-G5 is expressed by extravillous cytotrophoblast and erythroid cells. Paraffin-embedded sections of first-trimester trophoblast tissue were stained with commercial anti-HLA-G5 5A6G7 mAb, non-commercial 5A6G7 mAb and pan-HLA-G 4H84. Red staining indicates positivity. ER, erythroid cells; CIT, cell island trophoblast, i.e. extravillous cytotrophoblast.
and not the mode of production, since to date shed HLA-G1 and HLA-G5 have been shown to have similar inhibitory properties.

To date, the most relevant clinical implication of soluble HLA-G titration concerns IVF treatment: human IVF embryo secretes soluble HLA-G, and high secretion levels in the early cleavage stage are associated with the efficiency of embryo implantation (Fuzzi et al., 2002; Hviid, 2004; Sher et al., 2004, 2005; Warner et al., 2004; Noci et al., 2005; Yie et al., 2005). These data were obtained by multiple investigators and total more than one thousand samples and counting. Blaschitz et al. state that these results are controversial, but actually there is only one twelve-sample study that showed no HLA-G secretion by preimplanted embryos (Van Lierop et al., 2002).

References


loss of HLA-G1 expression and sensitivity to NK lysis. International J Cancer 00,00–00.