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Placenta-Derived Soluble MHC Class I Chain-Related Molecules Down-Regulate NKG2D Receptor on Peripheral Blood Mononuclear Cells during Human Pregnancy: A Possible Novel Immune Escape Mechanism for Fetal Survival

Lucia Mincheva-Nilsson,* Olga Nagaeva,* Ting Chen,* Ulf Stendahl,† Julia Antsiferova,* Ingrid Mogren,* Jenny Hernestål,* and Vladimir Baranov§

Mammalian pregnancy is an intriguing immunological phenomenon where the semiallogeneic fetus is not rejected. Tolerance toward the fetus involves a number of mechanisms associated with modifications of the immune status of the mother. In this study, we strongly suggest a novel mechanism for fetal evasion of maternal immune attack, based on the engagement and down-regulation of the activating NK cell receptor NKG2D on PBMC by soluble MHC class I-chain-related proteins A and B (collectively termed MIC). A similar immune escape pathway was previously described in tumors. We found that MIC mRNA was constitutively expressed by human placenta and could be up-regulated upon heat shock treatment. Our immunomorphologic studies showed that the MIC expression in placenta was restricted to the syncytiotrophoblast. Immunoelectron microscopy revealed a dual MIC expression in the syncytiotrophoblast: on the apical and basal cell membrane and in cytoplasmic vacuoles as MIC-loaded microvesicles/exosomes. Soluble MIC molecules were present at elevated levels in maternal blood throughout normal pregnancy and were released by placental explants in vitro. Simultaneously, the cell surface NKG2D expression on maternal PBMC was down-regulated compared with nonpregnant controls. The soluble MIC molecules in pregnancy serum were able to interact with NKG2D and down-regulate the receptor on PBMC from healthy donors, with the consequent inhibition of the NKG2D-dependent cytotoxic response. These findings suggest a new physiological mechanism of silencing the maternal immune system that promotes fetal allograft immune escape and supports the view of the placenta as an immunoregulatory organ. 

B glycoproteins are related cell surface molecules and are both highly polymorphic. The genes encoding these molecules contain heat shock response elements in their promoters (14, 15). MIC molecules are ligands of the NK-cell activating receptor NKG2D expressed on the surface of human NK, γδ T, and CDSαβ T cells (16). Normally, MIC proteins are constitutively expressed in low levels on epithelial cells in the gut and thymus, endothelial cells, and fibroblasts (14, 15), but become up-regulated or expressed de novo in stressed conditions, such as in tumors (12, 17), viral and bacterial infections (18–20), and in autoimmune conditions (21).

Although related to MHC class I Ags, MIC molecules and their murine homologs RAE/H60 do not present Ags, but serve as signals of cellular stress, and trigger a range of immune effector functions such as cytotoxicity and cytokine production (14, 22). The cross-linking of the “inducible-self” MIC molecules with the NKG2D receptor enables immune cells to recognize and attack stressed cells without the need of MHC class I expression or Ag recognition (23). Thus, the MIC/NKG2D interaction is an effective mechanism for immunosurveillance.

Several reports have shown that soluble form of MIC (sMIC) proteins is released from some types of tumors in the serum of cancer patients and affect NKG2D function by acting as competitive inhibitors that block the recognition of membrane-bound MIC molecules and down-regulate the NKG2D surface expression on NK and T cells (24–27). Thus, MIC molecules are not only involved in boosting cytotoxicity but also in its suppression. In this respect, they can be regarded as truly immunomodulatory molecules of the cytotoxic response.

Prompted by these findings, we set out to examine whether MIC proteins are expressed in human placenta, and whether a similar escape mechanism based on interaction between sMIC molecules and NKG2D receptor operates in pregnancy.

Materials and Methods

Samples, cell lines, and materials
Healthy women undergoing elective termination of pregnancy (8–14 wk of gestation) and at normal delivery donated placenta specimens after ethical committee approval and informed consent. The following Abs were used: affinity purified goat anti-MICA/B Ab (E-16) against a peptide mapping near the N terminus of MICA (Santa Cruz Biotechnology); anti-MICA mAbs from Immatics, AMO1 (specific for α1 and α2 domains of MICA), and BAMO3 (specific for the α3 domain of MICA and MICB), anti-NKG2D mAb, clone 149810 (R&D Systems), and clone 1D11 (BD Biosciences). Isotype-matched Ig control DAK-GO1 was purchased from DakoCytomation. Recombinant MICA was purchased from Immatics. The human MIC-expressing leukemia cell line K562 (28) was used as a target in cytoxicity assays.

Immunohistochemistry

Early and term placenta samples were fixed in 4% paraformaldehyde for 2 h, washed in PBS with 3.5% sucrose and 1% fish gelatin, and snap-frozen in liquid nitrogen. Cryosections (5 μm) were stained using goat anti-MICA/B Ab as described (9). Normal goat serum was used in all blocking steps. Parallel sections were stained after pretreatment with blocking peptides. Parallel sections were stained after pretreatment with blocking peptides.

Immunoelectron microscopy

Early and term placenta samples were fixed in 4% paraformaldehyde (Merck) in 0.1 M phosphate buffer for 4 h, washed in the same buffer containing 7% sucrose and 0.05% saponin (Merck) at 4°C overnight, and snap-frozen. Cryosections were processed by the indirect immunoperoxidase method (9) using goat anti-MICA/B Ab. Ultrathin sections were examined without additional staining. Sections incubated with PBS instead of specific Ab were used as negative controls.

Isolation of PBMC and trophoblast cells from first trimester placenta

PBMC from pregnant women and nonpregnant donors were isolated by Lymphoprep (Nycomed) gradient centrifugation.

First trimester trophoblast cells were isolated following the procedure described in Ref. 9 and used for total RNA preparation. For stress induction of MICA and MICB mRNA, the isolated cells were incubated at 42°C for 0, 30, and 60 min.

Short-term cultures of explants from early placenta

First trimester placenta samples (11–12 wk of gestation, n = 3) were cultured in vitro using a standard technique (29). Briefly, chorionic villi were washed with sterile RPMI 1640 and cut into small pieces of ~5 mg of wet weight. Two such pieces were cultured in individual tissue culture inserts (10-mm diameter, 0.4-μm polycarbonate mesh; Nunc) in 1.5 ml of culture medium (RPMI 1640, 10% FBS, and antibiotics). The explant preparations were performed in triplicates to assess reproducibility within the same placental sample. Cultures were maintained at 37°C in 5% CO2 and humidified air. Because syncytiotrophoblast viability in cultured human placentals is limited to 24 h (29), supernatants were harvested after 24 h of culture. Each supernatant was centrifuged at 400 × g and the presence of sMIC molecules was assayed by ELISA.

Total RNA extraction and quantitative real-time RT-PCR

Total RNA and cDNA were obtained as described (6) and amplified on ABI PRISM 7700 by TaqMan Gene Expression Plate (I) protocol (Applied Biosystems). Primer and probe sequences, detecting all allelic forms of MICA and MICB, were as follows: forward primer for both MICA/B, 5’-TGGAAACACAGGGGAATCA-3’; specific reversed primers for MICA, 5’-TGACTCTGAAACGGACGACT-3’; and MICB, 5’-TGACTGGAACGACGGCG-3’; and MICA/B probe, 5’-CACCACCTCGTGCCCTCTTGGGAA-3’; 18S was used as an endogenous control.

Cycling conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 5 s, and 60°C for 1 min. The MIC mRNA expression was presented as quantities related to a reference (=1) of MIC mRNA expression in PMA-ionomycin-stimulated PBMC (30). Capture ELISA for sMICA/B

ELISA plates (Nunc) were coated with goat anti-MICA/B Ab at a 1/100 dilution overnight. After blocking and washing, standards of rMICA, test sera and PBS were incubated for 1 h at room temperature. After washing, a mixture of anti-MICA/B mAbs AMO1 and BAMO3 was applied for 1 h followed by incubation with peroxidase-conjugated rabbit anti-mouse IgG (DakoCytomation) at 1/1000 for 1 h. Washed plates were developed by the TMB Peroxidase Substrate System (KPL), and absorbance was measured at 450 nm. The same ELISA procedure was used to determine sMIC concentration in culture medium of early placenta explants.

Immunoflow cytometry

NKG2D expression on PBMC from pregnant and control women was analyzed by flow cytometry with anti-NKG2D mAb (clone 149810) and quantified by comparing the mean fluorescence intensity (MFI) of the NKG2D staining. For blocking experiments of NKG2D expression, PBMC from healthy donors were incubated in 5% CO2 at 37°C for 24 h in the presence of pregnancy serum, pregnancy serum preincubated with anti-MICA/B Ab (10 μg/ml), serum from nonpregnant women, and medium alone. After incubation, the cells were stained for NKG2D as described above. In parallel, anti-CD3 staining was performed as a control of the specificity of the NKG2D blocking. A part of the cells incubated with pregnancy serum was fixed and permeabilized before staining. The fixation was done in 1.5% paraformaldehyde and 0.1% saponin for 20 min at room temperature. After washing, the aldehyde groups were blocked with 50 mM glycine in PBS for 15 min at room temperature. After centrifugation, the cells were resuspended in PBS with 0.1% saponin and allowed to permeabilize for 15 min at room temperature. Appropriate isotype controls were used. Five individual pregnancy sera from the first (n = 2) and second (n = 3) gestational trimester with the highest concentrations of sMICA as estimated by ELISA were tested in the blocking experiments.

Cytotoxicity assay

Cell-mediated cytotoxicity was measured by Live/Dead Viability Cytotoxicity kit (Molecular Probes Life Technologies). K562 target cells were labeled with the green fluorescence stain DiOC(3) for 20 min at 37°C. After labeling, the target cells were mixed with the effector cells at E:T ratio of 20:1 or 40:1, counterstained by propidium iodine (PI), pelleted.
and incubated in 96-well plates at 37°C in a humidified 5% CO₂ incubator for 2 h. Two-color scatter plot analysis separated live target cells (DiOC18, PI/H11001, P1/H11002) and membrane-compromised target cells (DiOC18, PI/H11001, P1/H11001). The percent-specific lysis was calculated by a standard formula according to the manufacturer’s instructions. For blocking of the NKG2D receptor, the effector cells were preincubated with pregnancy serum, normal human serum, rMICA, or blocking anti-NKG2D mAb (clone 1D11) at a concentration of 10⁻⁹/μg/ml. For blocking of the surface MIC molecules, the target cells were incubated with goat anti-human MICA/B Ab.

Statistics
We used the two-tailed Student t test for statistical analyses. A value of p < 0.05 was considered significant.

Results
Constitutive expression of MIC molecules in human placenta
Isolated trophoblast cells of early pregnancy constitutively expressed mRNAs for MICA and MICB, and could be up-regulated by heat shock treatment in a time-dependent manner as detected by real-time RT-PCR (Fig. 1, A and C). Term placenta samples expressed mRNA for MICA at a similar level as early placenta, while MICB mRNA was expressed at lower levels (Fig. 1B). To confirm the constitutive gene expression at the protein level, we stained samples from early and term placenta with goat Ab that recognize both MICA and MICB. To our knowledge, there are no commercial mAbs presently available that work in immunohistochemistry and discriminate between MICA and MICB. A positive staining was revealed in STB of early and term placenta (Fig. 2). Additionally, a weak staining of the wall of the blood vessels in the chorionic villi was observed (data not shown).

FIGURE 1. Constitutive expression (□) and heat shock up-regulation (■) of MICA and MICB mRNAs in human placenta. Quantitative real-time RT-PCR for MICA and MICB mRNA expression in freshly isolated and heat-shocked trophoblast cells from five early (A) and four term (B) placenta samples. C, Kinetic experiments showing time-dependent up-regulation of MICA and MICB mRNAs from early placenta (□).

FIGURE 2. Expression of MIC proteins in early and term placenta. MICA/B-positive staining of STB in early (A) and term (C) placenta. Insets: STB staining at a higher magnification (long arrow). CTB does not stain for MICA/B (short arrow). Negative controls: early (B) and term (D) placenta. Original magnification: A–D, ×400; insets, ×800.
Immunoelectron microscopy reveals a bipolar cell surface and intracellular expression of MIC molecules in STB

We determined the precise location of MIC molecules by immunoelectron microscopy (Fig. 3). There were no significant differences in the intensity of immunostaining and distribution pattern of MIC molecules in different villi from one placenta or among the three individual early placentas examined. The presence of MIC proteins was restricted to STB, confirming previous data that isolated CTB cells did not express MIC molecules (31). STB revealed a bipolar labeling along the apical and basal surfaces (Fig. 3, A–C).

It was previously shown that apical MIC expression in polarized epithelial cells is associated with MICA*008/A5.1 allelic combination, i.e., the most common in several populations (32). A Swedish investigation determined that the frequency of this MIC allelic combination was 71% (33). Basolateral MIC staining has been described earlier in the intestinal epithelium (34). Additionally, numerous vacuoles in the syncytioplasm were stained for MIC molecules (Fig. 3C). These vacuoles were ~1.5–2 μm in size and had variable cell locations (Fig. 3, C–E). Some of them fused with the apical plasma membrane and formed an opening to the intervillous space (Fig. 3D), others were in intimate contact with the nuclei (Fig. 3E). At high magnification, the vacuoles had a multivesicular ultrastructure, displaying numerous stained intraluminal microvesicles of 60- to 90-nm diameter (Fig. 3F). In some areas of the syncytioplasm, the rough endoplasmic reticulum (RER) and the Golgi complexes were stained (Fig. 3G). Similar results were obtained when term placenta was stained (data not shown). Negative controls, omitting anti-MIC Abs revealed no detectable MIC staining (data not shown). Two conclusions could be drawn from our electron microscopy studies: 1) MIC molecules were synthesized and expressed on the apical and basal surfaces of STB; and 2) MIC molecules were also present in numerous syncytioplasmic vacuoles, structurally similar to multivesicular bodies/secretory lysosomes (35, 36).

Soluble placenta-derived MICA/B molecules are present in the blood serum of pregnant women

The expression of MIC molecules by placenta implied the presence of STB-derived sMIC molecules in the maternal circulation. To test this suggestion, we performed a capture ELISA for sMIC

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**FIGURE 3.** Immunoelectron microscopy of early placenta stained by an indirect immunoperoxidase method without additional counterstaining. A, Low-power micrograph of STB and adjacent CTB. The electron-dense reaction product stains the apical microvillous surface (arrows) and the basal membrane (arrowheads) of STB. B, Micrograph of a basal part of STB showing positive staining of the basal membrane (arrowheads) and cytoplasmic processes (arrows) those penetrate between two CTB cells (+). BL, basal lamina. C, Micrograph demonstrating positive staining of the apical microvillous surface membrane (arrowheads) and cytoplasmic vacuoles (arrows). D, Micrograph of an apical portion of STB showing two labeled cytoplasmic vacuoles (arrows), one of which (thick arrow) forms an opening to the intervillous space (+). Arrowheads indicate stained microvilli. E, Micrograph of a perinuclear area of STB demonstrating a number of positively stained cytoplasmic vacuoles (arrows). N, nucleus. F, High-power micrograph of a cytoplasmic vacuole, containing a granular material and numerous MICA/B-positive internal microvesicles (arrowheads). G, High-power micrograph demonstrating MICA/B staining of the Golgi complex (arrows, +). Arrowheads point to labeled ribosomes of the RER. Magnification: A, ×3,500; B, ×9,100; C, ×8,900; D, ×15,700; E, ×15,400; F, ×17,000; G, ×17,500.
proteins (Fig. 4B) using sera from pregnant women in the first (8–11 wk, \( n = 12 \)), second (17–20 wk, \( n = 11 \)), and third trimester (33–38 wk, \( n = 7 \)); and nonpregnant sera \( (n = 5) \) shows significantly increased amounts of sMICA/B compared with controls. ROD, relative OD.

Explants from early normal placenta release sMIC proteins

To confirm that the sMIC molecules present in the serum of pregnant women are placenta-derived, explants from early normal pregnancy placentae of approximately the same gestational age (11–12 wk) were cultured for 24 h. The supernatant from the individual explant cultures was collected and analyzed for presence of sMIC by ELISA. The results from the three placental samples tested are summarized in Table I. As can be seen, all individual placenta released sMIC molecules at similar levels.

Reduced surface expression of NKG2D on PBMC from pregnant women

Given the previous finding of sMIC-induced down-regulation of NKG2D on effector cells (24, 27), we next investigated the expression of NKG2D receptor on PBMC from pregnant women \( (n = 9) \). The MFI of NKG2D expression was markedly reduced by \( \sim 55\% \) \( (p = 0.005, \text{Fig. 4A}) \) compared with nonpregnant controls. The decrease was not selective for a particular subset of PBMC, but was observed on NK, γδ T, and CD8^+ CB T cells (not shown), indicating a general down-regulation of NKG2D.

Soluble MIC molecules in pregnancy serum inhibit the surface NKG2D expression on PBMC from healthy donors

To test whether sMIC molecules but not other soluble factors were responsible for the reduced NKG2D surface expression on PBMC of pregnant women, we isolated PBMC from healthy donors \( (n = 5) \) and assessed the NKG2D expression before and after incubation with individual pregnancy serum, normal serum, or individual pregnancy serum, pretreated with MICA/B Ab. The results of one representative experiment are shown in Fig. 5. NKG2D surface expression declined 8- to 10-fold after incubation with pregnancy

![FIGURE 4.](https://example.com/figure4.png)

**FIGURE 4.** NKG2D on PBMC of pregnant women is down-regulated by sMIC proteins. A, Immunoflow cytometry of NKG2D-stained PBMC from pregnant women shows significantly decreased MFI compared with nonpregnant controls \( (n = 9, p = 0.004) \). B, Capture ELISA for sMICA/B with pregnancy sera from first (8–11 wk, \( n = 12 \)), second (17–20 wk, \( n = 11 \)), and third trimesters (33–38 wk, \( n = 7 \)); and nonpregnant sera \( (n = 5) \) shows significantly increased amounts of sMICA/B compared with controls. ROD, relative OD.

![FIGURE 5.](https://example.com/figure5.png)

**FIGURE 5.** Pregnancy serum down-modulates NKG2D expression on PBMC from healthy donors by ligand-induced endocytosis. Green-colored histograms represent NKG2D staining of untreated PBMC and gray-colored histograms represent negative control. Filled red histograms represent PBMC incubated with (A) pregnancy serum, (B) pregnancy serum, blocked with MICA/B Ab, (C) nonpregnant serum, and (D) PBMC incubated with pregnancy serum, fixed, and permeabilized before staining. Blue-colored histograms show anti-CD3 staining.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>No. of Explants</th>
<th>Approximate Wet Weight/Explant (mg)</th>
<th>sMIC Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>10</td>
<td>15.5 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>10</td>
<td>25.4 ± 8.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>10</td>
<td>20.0 ± 4.8</td>
</tr>
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serum compared with PBMC incubated with normal serum or untreated PBMC (Fig. 5, A and C). In contrast, the surface staining of CD3 remained unchanged. When pregnancy serum was pretreated with MICA/B Ab, the NKG2D expression was not down-regulated (Fig. 5B), suggesting that sMIC molecules were the main factor that reduced cell surface NKG2D expression. Pregnancy sera with the highest sMIC concentration were chosen from first and second gestational trimester and used individually in the blocking experiments. There was a tendency of higher down-modulation of NKG2D when the PBMC were treated with serum samples with higher sMIC concentration (data not shown). To determine whether NKG2D was internalized or just masked by the soluble ligand, a part of the PBMC that was incubated with pregnancy serum was fixed, permeabilized, and analyzed for the presence of intracellular NKG2D. The staining, reflecting the total NKG2D expression, showed MFI comparable or slightly reduced to that of freshly isolated PBMC, indicating that the cell surface down-modulation of NKG2D was caused by sMIC-induced endocytosis (Fig. 5D). These results are in contrast to the previously published data showing strong reduction of total NKG2D expression after ligand engagement in T cell clones (24) but in line with the results of Ogasawara et al. (37) demonstrating that the total amount NKG2D remained unchanged. The reason for these discrepancies cannot be ascertained from our experiments.

**Pregnancy serum down-regulates the in vitro NKG2D-dependent killing ability of PBMC from healthy donors**

Next, we assessed whether the NKG2D down-regulation by sMIC on PBMC had functional consequences for their killing ability (Fig. 6). The MIC-expressing target cells K562 were used at T:E ratio of 40:1 and 20:1. The PBMC were used directly, or after pretreatment with sMIC containing pregnancy serum, normal serum, NKG2D blocking mAb, or rMICA. Additionally, the target cells were pretreated with anti-MICA/B Ab to see whether blocking of sMIC molecules could affect the specific cytotoxicity. In the presence of pregnancy serum, the lysis of target cells was substantially decreased. The reduction of the specific lysis was similar to that seen when the effector cells were pretreated with anti-NKG2D mAb or with rMICA, or when MICA/B Ab blocked the MIC molecules on K562 cells. Normal human serum did not reduce cytotoxicity. These results show that down-regulation of NKG2D by sMIC molecules in pregnancy serum impairs the in vitro cytotoxic ability of PBMC.

**Discussion**

Four main points summarize the results presented here: 1) MIC molecules are constitutively transcribed in human placenta throughout normal pregnancy and sMIC molecules are released from in vitro cultured placental explants. 2) The MIC protein expression is restricted to STB, and exists in two forms—as a membranial form at the apical and basal surfaces, and as microvesicles in cytoplasmic vacuoles. 3) The cell surface expression of NKG2D receptors is significantly down-regulated on PBMC from pregnant women. 4) Elevated levels of sMIC molecules are present in pregnancy sera and are able to down-regulate the NKG2D receptor and impair the cytotoxic function of PBMC from healthy donors. Taken together, these results suggest a novel mechanism for immune evasion of the fetal allograft through fetal MIC and maternal NKG2D interactions.

The cell surface expression of MIC molecules on STB exposes the fetoplacental unit to maternal cytotoxic attack. It would make little sense, from the point of view of the fetus, to display molecules on the placenta that would identify it as abnormal and provoke rejection by the maternal immune system unless it gives the fetus an advantage. The permanent secretion/shedding of MIC molecules that we found in pregnancy explains this paradox. A similar mechanism of sMIC shedding is used by MIC-expressing tumors as immune escape strategy (24–27, 38). It has been shown that sMIC molecules shed by these tumors block NKG2D and impair the cytotoxic function of PBMC in cancer patients. In analogy with tumors, sMIC molecules present in pregnancy serum were able to down-regulate the NKG2D surface expression on PBMC and their killing ability. Placenta may be compared with a tumor: highly proliferative and invasive trophoblast cells penetrate the decidua and the uterine vasculature to build the placenta. These cells, like tumor cells, use similar molecular mechanisms for growth and migration. In both instances, changes in expression of adhesion molecules, proto-oncogene products and matrix-digestive enzymes occur, ensuring a rich blood supply (39, 40). Thus, human placenta can be regarded as a physiological counterpart of a tumor, and might use similar immune escape strategies. Alternatively, tumors may use evolutionary ancient immune evasion mechanism primarily used by placenta. Except in tumors, expression of cell surface and sMIC has been observed in a variety of pathological conditions such as viral and bacterial infections, inflammation, and autoimmunity (18–21). To our knowledge, pregnancy is so far the only example of a normal physiological condition that takes advantage of this adverse phenomenon and uses it to promote fetal allograft survival. However, the cost of using this escape mechanism might be the impairment of systemic maternal immune responses due to down-regulation of the NKG2D receptor and might be accountable for the observed fact that pregnant women are more sensitive to infections.

Very little is known about the intracellular localization or trafficking of MIC molecules (34). Our electron microscopy data throw some light on these events. The localization of MIC proteins in the RER and Golgi complex indicates their site of synthesis and maturation in STB. However, the major intracellular protein fraction was localized in numerous cytoplasmic vacuoles filled with MIC-positive microvesicles. There are two options for the identity of the MIC-containing vacuoles: they may either represent endocytosed microvesicle-associated complexes or newly synthesized MIC molecules in the so-called secretory lysosomes (41). We favor the latter explanation for the following reasons: 1) the multivesicular body-like structure of the vacuoles and the size of the microvesicles classify them as secretory lysosomes, i.e., organelles used for temporary storage of secreted proteins (41). 2) We used...
anti-MICA/B Ab directed to the extracellular domain of MIC molecules, thus, because the Ab stained intracellularly, they would be recognizing newly synthesized molecules that come directly from the synthetic pathway. If the vacuoles contained processed MIC proteins, i.e., endocytosed from the surface, the extracellular domain would have been cleaved by matrix metalloproteinases (MMP; Ref. 25), and we would have failed to stain the microvesicles. Therefore, the MIC-loaded microvesicles represent storage of newly synthesized protein in secretory lysosomes. The vacuoles were localized at different levels in the syncytioplasm, some reaching out and fusing with the apical surface membrane of STB. It has been shown that secretory lysosomes can fuse with the plasma membrane, resulting in release of the internal microvesicles into the external milieu. The released vesicles are then called exosomes (42). Convincing data indicate that intracellular microvesicles and exosomes are one and the same entity (43). Thus, we can suggest a release of MIC-containing exosomes from the placental villi to the maternal blood. This suggestion is supported by a recent report that MIC molecules were found in exosomes isolated from the growth medium of tumor cells and were able to impair cytotoxicity of NKG2D-positive effector cells (44). Exosome excretion is not only a well-known feature of tumor cells (45), but also a feature of the placenta, and is proven to be the form by which FasL is secreted by STB in the maternal circulation (8, 9). The alternative to exosomal MIC release is MMP-mediated shedding that has been proposed as a source of sMIC molecules in cancer patients (26). Because human placenta expresses high amounts of a variety of MMP (46), we cannot exclude the possibility that surface-cleaved MIC could also be a part of the ELISA-detected sMIC molecules. Thus, the serum MIC moiety in pregnant women might constitute a heterogeneous population of MMP-cleaved-, and exosome-released MIC molecules. Experiments are in progress to elucidate this assumption.

The immune escape mechanism described here concerns human normal pregnancy. An obvious question is how the MIC/NKG2D system works in pathological pregnancies threatened by abortion. We could up-regulate MIC mRNA expression in isolated villous trophoblast cells in vitro by thermal stress. Pathological conditions might constitute a heterogeneous population of MMP-cleaved-, and surface-cleaved MIC could also be a part of the ELISA-detected sMIC molecules. Experiments are in progress to elucidate this assumption.

Disclosures

The authors have no financial conflict of interest.

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