BASIC SCIENCE: OBSTETRICS

Maternal microchimerism in human fetal tissues

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OBJECTIVE: The aim of the present study was to analyze the presence of maternal cells in human fetal tissues in the second trimester.

STUDY DESIGN: Tissues from 11 second-trimester fetuses terminated because of social reasons or because of malformations and/or trisomy were investigated. By cell sorting and polymerase chain reaction amplification, we studied the presence of maternal CD3+, CD19+, CD34+, and CD45+ in different fetal tissues and in placenta.

RESULTS: In the group of fetuses with normal karyotype and normal autopsy findings, 4 of 5 fetuses were positive for maternal microchimerism. In the group in which the fetuses were diagnosed with trisomy 21 and/or malformations, we found cells of maternal origin in 3 of 6 fetuses.

CONCLUSION: The results from this study indicate that maternal microchimerism is a common phenomenon in the second-trimester fetuses. Maternal cells of lymphoid and myeloid lineages and hematopoietic progenitors are widely distributed in the second-trimester fetuses.

Key words: fetus, immunology, microchimerism, pregnancy

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The presence of a small amount of cells or deoxyribonucleic acid (DNA) that is derived from a genetic different individual is referred to as microchimerism. In pregnancy reciprocal cell traffic over the placenta may give rise both to fetal microchimerism in the mother and maternal microchimerism in the fetus.[1,2] Fetal cell trafficking to the mother seems to be a common phenomenon, and it has been studied extensively.[3-8]

Conversely, microchimerism of maternal origin has gained less attention. In cord blood, presence of maternal cells is a rather common phenomenon, and various detection rates (2-100%), depending on method have been reported.[9-12] It is unknown when during gestation maternal cells are passing over to the fetus, but cells of maternal origin have been detected in various concentrations in fetal blood from week 13.[13] In later life some of these cells seem to persist, and maternal cells have been detected in healthy subjects up to the age of 46 years.[14]

Information about maternal cells in various human fetal tissues is scarce. We reported recently on presence of maternal cells in tissues of a second-trimester fetus with malformations.[15] Interestingly, some of these cells expressed cell surface markers of hematopoietic stem cells. Srivatsa et al[16] detected maternal cells in various organs in 4 infants who died within the first week of life. It has been speculated that maternal microchimerism may be related to the origin of several disorders during childhood such as severe combined immunodeficiency,[17] juvenile dermatomyositis,[18,19] pityriasis lichenoides,[20] neonatal lupus syndrome,[21] and biliary atresia.[22] These studies are difficult to evaluate because there is a lack of information on maternal microchimerism in normal children and fetuses with regard to this aspect.

The aim of the present study was to further investigate the presence of maternal cells in different organs of second-trimester fetuses and to characterize the subset of maternal cells in fetal tissues.

Materials and Methods

Fetuses

Tissue samples were collected from 5 human fetuses (cases 1-5) in which the pregnancy was terminated in the second trimester (weeks 14-17) for social reasons. Another 5 cases of termination of pregnancy from the second trimester (cases 6-10) with trisomy 21 and/or malformations were investigated (weeks 15-18). A dichorionic twin pregnancy (cases 9 and 10) with trisomy 21 in both twins was included. Finally, a case of intrauterine fetal death (IUFD) diagnosed in week 27 was examined (case 11). The cases are described in Table 1.

The gestational age of the fetuses was determined by ultrasound performed in the second trimester. The abortion was performed by oral administration of mifepristone 600 mg on day 1 and misoprostol 800 mg vaginally followed by 400 mg misoprostol orally every third hour until the abortion was completed on day 3. The IUFD was induced with 600 mg mifepristone followed by vaginal administration of gemeprost the following day.

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TABLE 1
Characteristics of mothers and fetuses

Case #	Gestational age, wks	Reason for abortion	Fetal weight, g	Fetal sex	HLA class mother/fetus	Fetal autopsy findings/diagnosis
1	14	Social	42	F	HLA-A*11,32/HLA-A*11,11	Normal
2	14	Social	109	M	DRB1*04,10/DRB1*04,16	Normal
3	15	Social	57	M	DRB1*14,15/DRB1*01,14	Normal
4	16	Social	89	M	DRB1*0103,04/DRB1*04,08	Normal
5	17	Social	119	M	HLA-B*39,51/HLA-B*18,51	Normal
6	15	Trisomy 21	62	F	DRB1*0403,10/DRB1*10,10	Cleft palate, micrognathia, pterygium colli, artrogryposis, cystic kidneys, hypoplastic ureter
7	17	Trisomy 21	94	F	DRB1*03,1501/DRB1*03,11	Hygroma colli, no internal malformations
8	18	Malformations	236	F	HLA-A*012601 /HLA-A*01,02	Lumbosacral meningomyelocele. Normal karyotype
9	18	Dichorionic twin pregnancy, trisomy 21	195	M	DRB1*0401,13/DRB1*11,13	Hygroma colli, ventricular septum defect, abnormal lung lobation
10	18	Dichorionic twin pregnancy, trisomy 21	206	M	DRB1*0401,13/DRB1*11,13	Hygroma colli, no internal malformations
11	27	Stillbirth	680	M	DRB1*03,1103/DRB1*03,15	Facial dysmorfism, no internal malformations

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The body of the fetus was properly washed prior to autopsy according to standard procedures. The fetuses were dissected under sterile conditions, and fetal tissues were collected and placed in sterile NaCl. Peripheral blood was collected from the mothers. The study was approved by the Ethics Committee at Huddinge University Hospital (Dnr 129/01).

Cell separation and DNA extraction

The fetal tissues were disintegrated by passage through a 100-μm nylon mesh to form a single cell suspension and diluted in NaCl. To evaluate lineage-specific chimerism in cases 1, 3, 4, 5, 8, 9, and 10 separations of CD3+ (T cells), CD19+ (B cells), CD34+ (hematopoietic progenitor cells), and CD45+ (leukocytes) cells derived from various organs of the fetuses were made by means of immunomagnetic beads according to the manufacturer's instructions (Dynal Biotech, Oslo, Norway). Each cell-separated pellet was lysed and prepared according to previously described method.[23] DNA was extracted from all tissue samples using a standard salting-out procedure. Genomic DNA concentrations were measured in a Gene Quant II spectrophotometer (Pharmacia, Uppsala, Sweden)

Human leukocyte antigen (HLA)-DR typing

HLA typing was performed by polymerase chain reaction (PCR) amplification with sequence-specific primers.[24] We started with HLA-DR and if the sequence difference was not large enough between the fetus and the mother, we also typed for HLA-A and HLA-B. Primers specific for maternal HLA sequences were designed for each fetus-mother pair. These primers were tested for specificity and sensitivity. The HLA differences we used for analysis are shown in Table 1.

Detection of maternal alleles in the human fetus by PCR amplification

PCR amplifications were performed with maternal-specific primers in a volume of 50 μL containing 1μg genomic DNA, 0.5 μM of each primer, 200 μM of each dNTP (Perkin-Elmer, Branchburg, CA), 1 × PCR buffer (1.5 mM MgCl, 50 mM Mg, 10 mM KCl, 0.001% gelatin), 5% glycerol, 5 μg cresol red, and 1.5 U AmpliTaq polymerase (Perkin-Elmer). After an initial 3 minute hot-start/denaturation step at 94°C, 40 PCR amplification cycles were carried out in a PTC-200 thermal cycler (MJ Research, Watertown, CA). The first 10 cycles were done in a 2-segment step at 94°C for 20 seconds and at 62°C for 1 minute. The fol-