Fetal Mesenchymal Stem-Cell Engraftment in Bone after In Utero Transplantation in a Patient with Severe Osteogenesis Imperfecta

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Background. Mesenchymal stem cells (MSC) are progenitors of mesenchymal tissues such as bone, cartilage, and adipose. Adult human leukocyte antigen (HLA)-matched MSC have been used in cellular therapies of bone disorders such as osteogenesis imperfecta, with promising results.
Methods. A female fetus with multiple intrauterine fractures, diagnosed as severe osteogenesis imperfecta, underwent transplantation with allogeneic HLA-mismatched male fetal MSC in the 32nd week of gestation. Engraftment analyses of donor cells, immunologic reaction against donor cells, and the well-being of the patient were assessed.
Results. At 9 months of age, on slides stained for osteocalcin or osteopontin, a centromeric XY-specific probe revealed 0.3% of XY-positive cells in a bone biopsy specimen. Whole Y genome fluorescent in situ hybridization staining showed a median of 7.4% Y-positive cells (range, 6.8%–16.6%). Bone histology showed regularly arranged and configurated bone trabeculae. Patient lymphocyte proliferation against donor MSC was not observed in co-culture experiments performed in vitro after MSC injection. Complementary bisphosphonate treatment was begun at 4 months. During the first 2 years of life, three fractures were noted. At 2 years of corrected age, psychomotor development was normal and growth followed the same channel, −5 SD.
Conclusions. The authors' findings show that allogeneic fetal MSC can engraft and differentiate into bone in a human fetus even when the recipient is immunocompetent and HLA-incompatible.

Keywords: Osteogenesis imperfecta, Mesenchymal stem cells, In utero transplantation, Fetal transplantation, Tolerance, Immunity.

(*Transplantation* 2005;79: 1607–1614)

In utero transplantation (IUT) with hematopoietic stem cells offers a possibility for curing fetuses with various congenital disorders (*1*). In humans, successful IUT has so far been limited to fetuses with severe immunologic defects, where there is a survival advantage for donor cells (*2–4*). Studies on the ontogeny of fetal immune development suggest that the human fetus can mount an alloresponse as early as the second trimester (*4, 5*). The presence of a functionally developed immune system at such an early stage of pregnancy calls for alternative strategies if IUT is to be a realistic therapeutic alternative.

Why perform IUT and not early postnatal transplantations? The rationale for IUT is based on the assumption that treatment before birth is preferable because the target disease is lethal for the fetus or will result in early childhood morbidity, making postnatal therapy less effective. Furthermore, common arguments for IUT are the lack of myeloablation requirement, the high proliferative capacity and exponential expansion of cellular compartments during fetal life, the normal migration of stem cells to different anatomic compartments and the greatly increased cell dosage given the size and weight of the fetus, and the far better psychosocial situation for the mother and father resulting from the birth of a child who has already been treated.

Adult bone marrow-derived mesenchymal stem cells (MSC) are not inherently immunogenic (*6–8*). They do not induce proliferation of allogeneic lymphocytes and escape lysis by cytotoxic T cells and alloreactive natural killer cells (*9*). In vivo, persistence of human MSC has been observed in multiple tissues of newborn sheep after human MSC infusions in utero even after immunologic competence had been established in the fetus (*10–14*). Instead, MSC appear to have immunomodulatory effects and suppress T-cell responses induced by mitogens and in mixed lymphocyte cultures (*6–8*,

This work was supported by grants from the Swedish Cancer Society (0070-B03-17XBC and 4562-B03-XAC), the Children's Cancer Foundation (03/039 and 03/007), the Swedish Research Council (K2003-32X-05971-23A and K2003-32XD-14716-01A), the Tobias Foundation, the Stockholm Cancer Society, the Swedish Medical Society, the European Commission Biomed 2 Programme (Eurofetus project), and Karolinska Institutet.

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Received 23 December 2004. Accepted 17 January 2005.

ISSN 0041-1337/05/7911-1607
DOI: 10.1097/01.TP.0000159029.48678.93

15, *16*). An immunosuppressive effect of MSC in vivo has been shown in a baboon model, where infusion of ex vivo expanded major histocompatibility complex (MHC)-mismatched donor MSC delayed the time to rejection of histoincompatible skin grafts (*15*).

Like adult bone marrow-derived MSC, fetal liver-derived MSC are nonimmunogenic in vitro (*17*, *18*). Both adult and fetal MSC retain multilineage potential and form cartilage, adipose, bone, and muscle, among other tissues, on induction (*17*, *19–21*). Several independent reports have confirmed that systemically infused human, rodent, and baboon MSC engraft in nonhematopoietic tissues and acquire the phenotype of the tissue they repopulate (*22–26*). These observations raised the possibility of cell-based therapy for genetic disorders of mesenchymal tissues.

Fetal liver-derived MSC have increased proliferative capacity compared with adult bone marrow-derived MSC (*18*). When cultured in vitro, the fetal MSC do not differentiate spontaneously, but after appropriate induction, they form adipose, cartilage, and bone, like their adult counterparts. Thus, we chose fetal MSC as the donor cell source for the intrauterine transplantation, hypothesizing that they propagate better than adult MSC in the fetal environment.

Osteogenesis imperfecta (OI) is a debilitating disorder resulting in the formation of brittle bones. Mutations in the *COL1A1* and *COL1A2* collagen genes perturb normal collagen fibril assembly (*27*, *28*). Several reports indicate a role for cell therapy in the treatment of OI. Infusion of wild-type MSC into OI-transgenic mice significantly increased the bone content of both collagen and mineral (*29*). Horwitz et al. investigated bone marrow transplantation in children suffering from severe OI and reported increased growth velocity, total body mineral content, and fewer fractures (*30*). In a later study, isolated MSC from the original bone marrow donors were gene-marked and infused into the patients (*31*). A low level of donor MSC was detected by polymerase chain reaction (PCR) and growth was accelerated. This suggests the safety and feasibility of MSC therapy and a potential benefit to children with OI. Encouraged by these reports, we used IUT of fetal MSC to treat a fetus with severe OI.

PATIENTS AND METHODS

Patient

A 26-year-old gravida I underwent an ultrasound examination in the 15th week of gestation. Femur length was below the fifth percentile. Amniocentesis revealed a normal female karyotype. Ultrasound examinations during weeks 24 to 27 showed that all the limbs were below the fifth percentile, with angulated and fractured femur bones. A tentative diagnosis of OI was made. In the 30th week, the responsible physician raised the possibility of an IUT with MSC. The parents chose this therapeutic option, although they were aware that the fetal treatment was experimental and that the outcome was uncertain. The transplantation procedure was approved by the Ethics Committee of Karolinska University Hospital (Dnr 91:157).

MSC Isolation and Preparation

MSC were isolated from one male fetal liver (10 weeks) aborted in the first trimester where the woman had volunteered to donate fetal tissue. The study was approved by the Ethics Committee at Karolinska University Hospital (Dnr 428/01) and written consent was obtained from the patient.

The cells were cultured as reported elsewhere (*17*). Briefly, the fetal liver was disrupted by passage through a 100-μm nylon filter and the cell suspension was diluted to a final concentration of 10^7 cells/mL. Mononuclear cells were collected by gradient centrifugation and suspended in Dulbecco's modified Eagle's medium-low glucose (Invitrogen, Paisley, Scotland) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were plated at 1.6×10^5 cells/cm^2 in culture flasks and maintained at 37°C in a humidified environment containing 5% carbon dioxide. After 3 days, nonadherent cells were discarded and the medium was replaced every 3 to 4 days thereafter. At near confluence, the cells were detached by treatment with 0.05% trypsin and 0.53 mM EDTA (Invitrogen) and replated to a density of 4×10^3 cells/cm^2 in culture flasks. At passage 2, the cells were harvested. Aliquots of cells were assayed for sterility, human immunodeficiency virus antigen, human T-cell lymphotropic virus types I and II, hepatitis B and C, and mycoplasma. Harvested cells constituted a single phenotypic population by flow cytometry, being uniformly positive for CD29, CD44, CD73, CD105, and CD166 and intermediately positive for human leukocyte antigen (HLA) class I. They were negative for HLA class II and the hematopoietic markers CD14, CD34, and CD45. The ability of the cells to differentiate along adipogenic, chondrogenic, and osteogenic lineages was assayed, as previously described in more detail (*17*). Harvested cells were counted, their viability was evaluated, and they were concentrated to a volume of 2 mL in sterile NaCl.

HLA Typing

Genomic DNA was extracted from proteinase K-treated recipient peripheral blood or cultured donor MSC by means of the salting-out method. A ready-to-use system consisting of sequence-specific primers (Olerup SSP, Saltsjöbaden, Sweden) was used to identify specific alleles at the HLA-A, HLA-B, HLA-C, and DRB1 loci for both donor and patient. PCR reactions were carried out in a PTC-200 thermal cycler (MJ Research, Watertown, CA) according to the manufacturer's recommendations. The results of HLA typing of the donor and recipient were as follows: donor MSC, HLA-A*24, B*15,*27, Cw*0103,*03, DRB1*07,*15; patient, HLA-A*03, B*15,*57, Cw*07,*18, DRB1*04.

Culture of Fibroblasts

Umbilical cord fibroblasts isolated at birth and skin fibroblasts isolated at 9 months of patient age were cultured in Ham's F-10 and RPMI 1640 media supplemented with 10% fetal calf serum (Sigma) and 20 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20 mM L-glutamine (Invitrogen).

Sequencing Assay

The complete coding sequence of *COL1A1* and *COL1A2* was amplified from genomic DNA prepared from umbilical cord and skin fibroblast cultures. Cycle sequencing protocols were based on the genomic primers described by Körkkö et al. (*32*).

Chimerism Studies

PCR amplifications on DNA extracted from umbilical cord fibroblasts harvested at birth, fibroblasts from a skin biopsy, and bone marrow-expanded MSC taken at 9 months of patient age were performed with two DRB1 donor-specific primers: DRB1*07 (5'-primer, 5'-ACGTTTCCTGTGGCAGGGTAAGTATA-3'; and 3'-primer, 5'-CCCCGTAGTTGTGTCTGCACAC-3') and DRB1*15 (5'-primer, 5'-GTTTCCTGTGGCAGCCTAAGAGG-3'; and 3'-primer, 5'-TCCACCGCGGCCCGCGC-3') in a volume of 50 μL containing 1 μg of genomic DNA in a PTC-200 thermal cycler (MJ Research). The PCR reactions started with an initial 2-min denaturing step and included 10 cycles (94°C for 10 sec, and 65°C for 60 sec) and 30 further cycles (94°C for 10 sec, 61°C for 50 sec, and 72°C for 30 sec). PCR products were separated on a ready-to-use polyacrylamide gel electrophoresis gel (12.5% nondenaturing polyacrylamide gel under standard buffer conditions at 10°C; Amersham Pharmacia Biotech, Uppsala, Sweden) for 1 hr and visualized by an automated silver staining method (Amersham Pharmacia Biotech). The band patterns were then analyzed in visible light.

Bone Histology Studies

A bone biopsy was taken at 9 months, embedded in paraffin, stained with hematoxylin-eosin, and examined morphologically. For immunohistochemistry, slides (4 μm) were dewaxed before incubation with one of three different bone markers: osteocalcin (rabbit–anti-human, 1:500; Cambio, Cambridge, United Kingdom), bone sialoprotein (BSP) (rabbit–anti-human, 1:1,000; Chemicon, Hampshire, United Kingdom), and osteopontin (mouse–anti-human, 1:500; MPIIIBIO$_1$). Nonspecific staining was determined by using corresponding isotype controls. Slides were washed and incubated with horseradish peroxidase-conjugated secondary antibodies, goat–anti-rabbit, and sheep–anti-mouse (DAKO, Glostrup, Denmark). DAB solution (DAKO) was used as a substrate for the peroxidase enzyme reaction, and positive staining was analyzed using light microscopy.

Fluorescent In Situ Hybridization

Osteocalcin-, osteopontin-, and bone sialoprotein-stained slides were scanned and photographed at magnifications of 20× and 100×. The same slides were subsequently prepared for fluorescent in situ hybridization (FISH) with a tissue pretreatment kit (Q-BIOgene, Illkirch, France). One of two sets of FISH probes: α-satellite probes for the centromeric regions of X (green) and Y (red) (Abbott-Vysis, Abbott Park, IL) or probes for the total human genome X (green) and Y (red) (Abbott-Vysis) was applied. To ensure the quality of the analysis, we hybridized iliac bone sections from healthy male and female controls with the same probes. The slides and the probes were simultaneously denatured in a HYBrite at 73°C and hybridized at 37°C for 16 hr. After washing, the slides were counterstained with VectaShield DAPI (4'6-diamidine-2-phenylidole dihydrochloride)/antifade (Vector Laboratories, Burlingame, CA) for detection of cell nuclei. They were scanned in a Nikon microscope and XY/XX cells were counted. In the osteocalcin-stained slides, 1,500 to 2,000 cells per section in four sections were counted, and in slides stained with osteopontin and BSP, an average of 500 to 1,000 cells were counted in four and five sections, respectively. Hybridization was greater than 95% in the centromeric XY probe-stained slides and 90% in slides stained with total genome X and Y probes.

Immune Response Assay

Blood samples for MLC were collected from the umbilical cord before transplantation and at birth, 7 months, and 9 months of patient age. Peripheral blood lymphocytes were prepared by centrifugation on a Ficoll gradient (Lymphoprep; Nycomed Pharma, Oslo, Norway). Triplicate samples of 1×10^5 lymphocytes were cultured with 10,000 irradiated donor or control MSC in 0.2 mL RPMI 1640 medium supplemented with 20 mM HEPES, 100 IU/mL penicillin, 100 μg/mL streptomycin, 20 mM L-glutamine (Invitrogen), and 10% pooled human AB serum in 96-well plates, to investigate whether the donor MSC induced an allograft reaction in the patient. The cultures were incubated at 37°C in humidified 5% carbon dioxide air for 6 days. On day 5, 1 μCi/mL ^{3}[H]-thymidine (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) was added. After 24 hr, the cells were harvested on a glass-fiber filter (Wallac, Turku, Finland) using a semiautomatic harvesting machine (Harvester 96; Tomec, Orange, CT). Radioactivity was determined as counts per minute with an Intertechnique β-counter (Wallac).

RESULTS

Transplantation of Fetal MSC and Clinical Course

The umbilical vein was punctured at week 32 under ultrasound guidance and analgesia of the fetus. Altogether, 6.5×10^6 fetal MSC with a viability of 90% were injected. There were no signs of fetal distress during the MSC injection, and the pregnancy was uncomplicated thereafter. At 35 weeks, after spontaneous preterm rupture of the membranes, the baby girl was delivered by cesarean section. Apgar scores were 9 and 10 at 1 and 5 min, birth weight was 1,669 g (<2 SD), length was 40 cm (−3 SD), and head circumference was 29 cm (−2.5 SD), typical of severely affected OI neonates (*33*). A frontal bossing and a soft calvarium were noted, and her rib cage was slightly asymmetric. Radiography showed wormian skull bones, generalized osteopenia and platyspondyly, and thin gracile bowed long bones with deformities indicating healed fractures and an actual fracture of the right femoral diaphysis. The femoral fracture was stabilized and the patient was discharged at 3 weeks' postnatal age. Because of osteopenia and new compression fractures of the spine, treatment with pamidronate was instituted at 4 months (*34*). Two fractures have been clinically suspected, a clavicular fracture at 6 weeks and a costal fracture at 9 months. A femoral fracture occurred at 15 months after a fall from 1 m. Healing of the fracture was prompt after initial stabilization with a bandage. Dual-energy x-ray absorptiometry of the lumbar spine (using the Hologic QDR 4500 system) revealed a skeletal mineralization of 48% that of age-matched controls at 3 months that increased to 56% at 12 months and 76% at 22 months, improvements at least partially attributable to pamidronate therapy. The 2 years of life were otherwise uncomplicated, without hospitalization. The first teeth erupted at 9 months. At 2 years, her psychomotor development was normal although it was small for her age, and her growth velocity

was similar to that for unaffected children, following the same −5 SD channel for weight and height. She wears orthopedic shoes to counteract the development of pes valgus and moves around without restrictions.

Sequencing Assay

In addition to several previously characterized polymorphisms, the patient was found to be heterozygous for a missense mutation (g33743 G/A, bases numbered according to AF004877) in exon 46 of the *COL1A2* gene (Fig. 1A). This substitution destroys a recognition site for the restriction enzyme Bgl I (NEB), and loss of this site was confirmed in two separate PCR products containing exon 46 in DNA from both umbilical cord and skin fibroblasts. This glycine-to-aspartic acid mutation at amino acid 1003 of the triple helix has not been previously reported in other OI patients; however, similar mutations are associated with a severe, nonlethal phenotype (Fig. 1B) (*35*, *36*).

Bone Histology

At 9 months, a bone biopsy showed regularly arranged and configurated bone trabeculae lined by a columnar layer of normal osteoblasts (Fig. 2). The amount and distribution of osteocytes and the ossification were normal for her age. There were no apparent signs of healing or remodeling. Cellularity was 100% in the marrow cavity, with no fibrosis present. All myeloid cell lines were represented and of normal morphology.

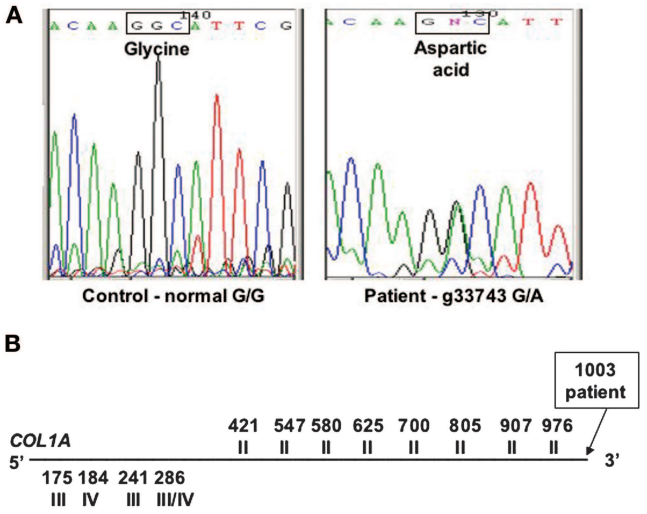

FIGURE 1. Mutation analysis of the patient's *COL1A2* gene. (A) Electropherograms of DNA from the patient and a normal control subject illustrating the region containing nucleotide 33743 of the genomic *COL1A2* sequence. The patient has an adenine-to-guanine substitution, resulting in a glycine-to-aspartic acid mutation. (B) The distribution and phenotype of reported glycine-to-aspartic acid changes in previously reported OI patients. Patients with type II OI are represented above the line, and those with milder phenotypes (III and IV) are shown below it. (Data are from the type I collagen mutation database: http://www/le.ac.uk/genetics/collagen/col1a2.html.)

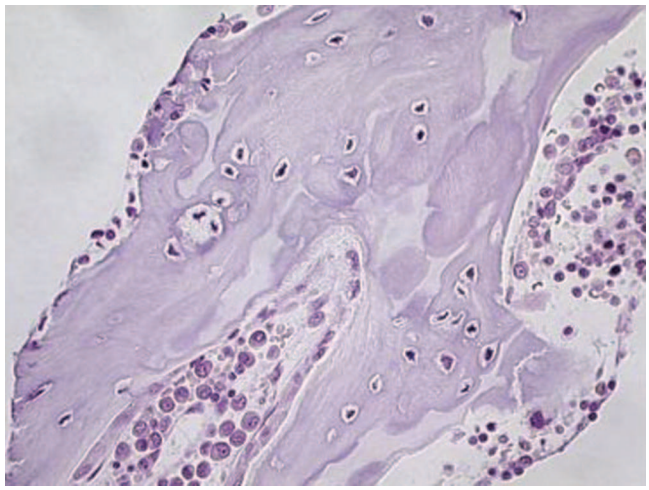

FIGURE 2. Hematoxylin-eosin staining of a bone biopsy taken at 9 months of age. Bone trabeculae were well-configurated and regular, and lined by a columnar layer of normal osteoblasts. The amount and distribution of osteocytes and the ossification were normal for this age.

Engraftment and Identification of MSC

A centromeric XY-chromosome–specific probe identified 17 cells (0.3%) containing XY in 6,000 cells stained with osteocalcin in a bone biopsy (Fig. 3A). In slides stained for osteopontin, four XY cells (0.3%) were detected in 1,600 cells studied with the centromeric XY probe. In BSP-stained slides hybridized with probes against the whole male and female genome, Y-positive cells similar to those observed in male control slides were detected in the patient but not in female controls. Using the whole male genome probe, we detected 228 Y chromosome-positive cells (median, 7.4%; range, 6.8%–16.6%) in a total of 2,500 cells stained with BSP (Fig. 3B). The probability of finding the centromeric region in sliced samples is lower than that of finding any region of the Y genome and could explain the lower number of Y-positive cells found with the centromeric-specific probes. A total of three triploid cells (XXY) and one tetraploid cell (XXXY) were identified in nearly 10,000 cells counted.

DNA isolated from umbilical cord fibroblasts harvested at birth and from skin fibroblasts and bone marrow MSC expanded in culture harvested at 9 months of age was analyzed by PCR. No donor DNA was detected.

Immune Response

Peripheral blood lymphocytes from the patient at the time of transplant proliferated against allogeneic lymphocytes in vitro in co-culture experiments, demonstrating immunocompetence of the fetus (Fig. 4A). However, alloreactivity was not detected against donor fetal MSC in vitro before transplant (Fig. 4A). Postnatally, lymphocytes isolated from the child at birth and at 7 and 9 months of age continued to respond to allogeneic lymphocytes in MLC (Fig. 4B–D). At none of the time points tested were lymphocytes from the recipient stimulated by the donor fetal MSC, indicating that no immune reaction had occurred against the transplanted cells (Fig. 4B–D).

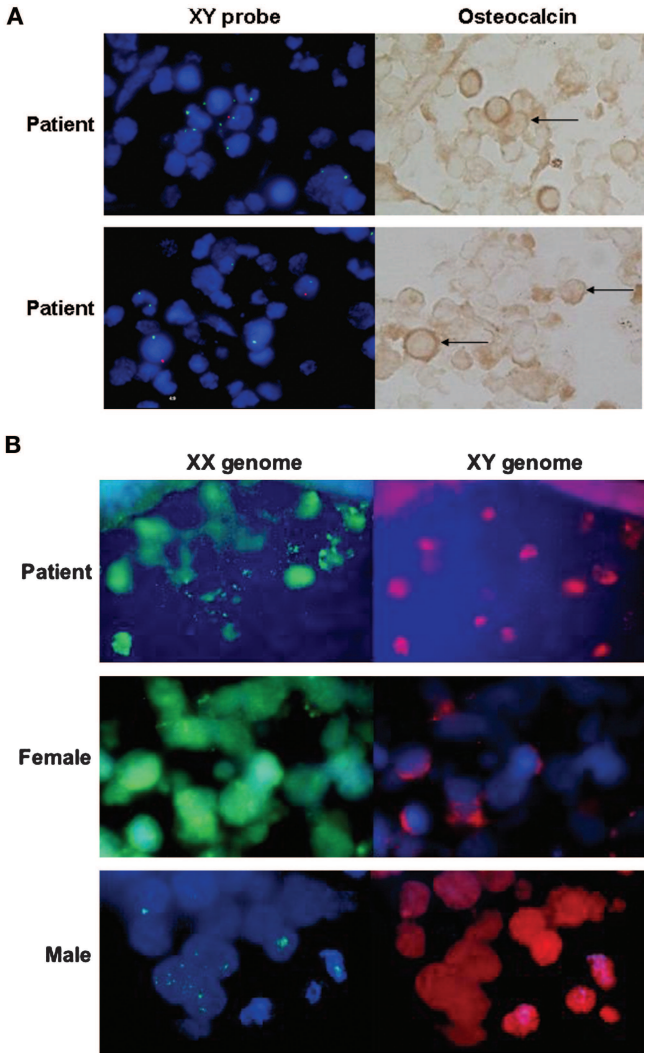

FIGURE 3. Detection of donor-derived cells in sections of a bone biopsy specimen obtained at 9 months of age. Analyses of 4-μm single sections by immunohistochemistry followed by X and Y chromosome FISH. (A) α-Satellite probes for the centromeric regions. Both X (*green dots*) and Y chromosomes (*red dots*) can be recognized in DAPI-staining nuclei of osteocalcin-positive cells. (*arrows*) Cells containing Y chromosomes. (B) Bone sections from the patient and healthy female and male controls were hybridized with probes for the total human genome X (*green*) and Y (*red*) with DAPI staining of the nucleus.

DISCUSSION

Our study demonstrates that allogeneic MSC of fetal liver origin can be transplanted across MHC barriers and, after intravenous transplantation in utero, undergo site-specific differentiation to bone and persist for extended times. Donor cell engraftment was 0.3% by analysis with the centromeric Y chromosome-specific probe. A considerably higher level of donor cell engraftment (7.4%) was estimated using the total Y chromosome painting probe, which recognizes multiple parts of the Y chromosome. Because osteocytes are terminally differentiated cells, the presence of osteocalcin-, osteopontin-, and bone sialoprotein-positive cells of male origin in bone more than 9 months after transplantation suggests that the transplanted cells participate in biologic turnover and provide a continual source of osteoblastic progenitor cells. From the time of transplant until the bone biopsy specimens were collected, the child grew appreciably. Because pamidronate treatment does not stimulate growth in OI children (*37*), the engrafted MSC may be partially responsible for this considerable growth (*31*), which is evidence of a functional contribution of the differentiated MSC to the osteogenic environment.

Previous reports indicate that prolonged culturing compromises the engraftment and differentiation capacity of adult MSC (*31*, *38*). The cells transplanted in the present study were harvested during their second passage and had not been expanded substantially ex vivo. However, it is possible that the fetal MSC, which exhibit a significantly higher proliferative capacity than adult-derived MSC, have an increased potential to generate large quantities of osteoblasts in the long term.

We could not detect donor cells by PCR on culture-expanded umbilical cord and skin fibroblasts or in a marrow aspirate expanded in vitro in MSC medium. The negative results for MSC derived from the bone marrow aspirate could be explained by the low sample volume. Also, detection of donor MSC has proved difficult with cultured bone marrow aspirates from both human and animals, because MSC are preferentially located in the endosteum (*25*, *31*, *39*). Another explanation is loss of proliferation potential by expanded MSC after intravenous infusion (*40*). Recently, Spees et al. reported that cell fusion, including nuclear fusion, was frequent in ex vivo co-cultures of adult MSC and heat-shocked small airway epithelial cells (*41*). We therefore looked for evidence of binucleated cells in sections stained with DAPI and for double FISH signal intensity from DAPI-stained nuclei, which would clearly reveal the presence of double or larger amounts of chromatin. In more than 10,000 cells examined, we found 4 polyploid cells.

This is the first demonstration of donor MSC engraftment in a human fetus with normal immunologic function. In vitro studies suggest that engraftment occurred because MSC are immune-privileged cells. Several reports have indicated that neither fetal nor adult MSC are inherently immunogenic and do not elicit proliferation of allogeneic lymphocytes in co-culture experiments (*6–8*, *15*, *16*, *42*). Adult MSC express MHC class I and contain intracellular deposits of HLA class II that can be increased on the cell surface after stimulation of the cells with interferon-γ for 1 or 2 days (*42*). The fetal MSC used here, and those derived from other first-trimester fetuses studied by our group, express HLA class I but require exposure to interferon-γ for 1 week for de novo synthesis and induction of cell surface expression of class II alloantigens (*18*). Interestingly, in spite of HLA class II expression, the cells do not elicit alloreactivity in vitro, suggesting that they possess unique immunologic properties. Several animal studies also indicate transplantability of MSC across MHC barriers. Infusion of mismatched human fetal MSC promotes the engraftment of umbilical cord blood-derived $CD34^+$ cells (*43*). Furthermore, MSC persist long term after allogeneic transplantation in baboons (*25*, *26*).

Encouraged by the available in vitro data, we hypothesized that fetal MSC would be transplantable between HLA-mismatched individuals. Before transplantation, prolifera-

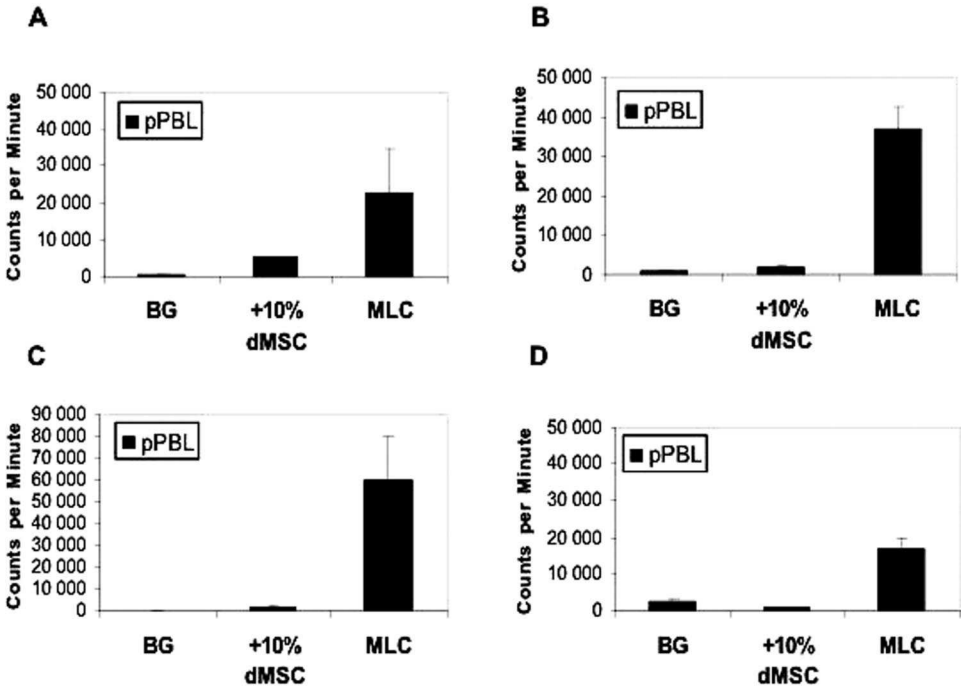

FIGURE 4. MLC with patient lymphocytes and donor MSC; 10,000 MSC or 100,000 allogeneic peripheral blood lymphocytes were co-cultured with 100,000 patient peripheral blood lymphocytes. (A) Before transplantation. (B) At birth. (C) At 7 months of age. (D) At 9 months of age. An immune response was detected against allogeneic lymphocytes but not donor MSC. The data are reported as mean±SD of three experiments.

tion of donor lymphocytes against allogeneic peripheral blood lymphocytes was detected in mixed lymphocyte cultures, confirming immunocompetence of the fetus. In contrast, alloreactivity against donor MSC was not detected before transplantation or when investigated at different times after transplantation. This indicates a lack of immune reactivity against the transplanted MSC; however, to demonstrate definitely a lack of alloresponse to the donor, one would need to test donor tissues other than MSC against the recipient lymphocytes. This was not possible because no other donor tissue was available.

OI is a heterogeneous disorder with clinical characteristics that include bone fragility and deformity, alterations in scleral hue, diminished stature, and decreased bone mineralization (*44*). The patient described here presented several features suggestive of a severe OI phenotype, including markedly diminished calvarial mineralization with wormian bones; intrauterine fractures of long bones; and angulation, platyspondyly, and blue sclerae. Most of the mutations that have been identified in OI in the *COL1A1* and *COL1A2* genes are single base changes that convert a codon for glycine to a codon for another amino acid (*45*). Genetic analysis of our patient revealed a mutation near the 3' end of the *COL1A2* gene, resulting in a glycine-to-aspartic acid substitution of the α2(I) procollagen triple helical domain, which is consistent with a severe, nonlethal phenotype (*35*, *36*). However, the severity of OI also depends on the relative balance between the rates of synthesis of mutated and normal pro-α polypeptide chains (*46*). In children with OI who have undergone transplantation with MSC from an HLA-identical sibling donor, the presence of only 1% to 2% donor MSC leads to clinical improvement (*31*). Thus, low levels of MSC engraftment may be sufficient to produce a shift in the balance between the synthesis of mutated and normal pro-α chains, thereby converting a severe OI phenotype to one that is less severe. In the patient described here, it was not possible to determine whether the low presence of donor-derived cells had any influence on the OI phenotype. Such assessment was complicated by the variability in OI severity and by complementary treatment with bisphosphonates, which may also have affected the clinical outcome. Controlled studies are needed to fully evaluate the therapeutic effect of donor MSC therapy.

The technical aspects of IUT also include the risk of procedure-related complications. In utero transplantation is usually performed with a 22-gauge needle and involves injection of a small volume. It is a rather simple ultrasound-guided procedure that lasts for only 5 to 10 min. From experience with invasive procedures such as cordocentesis and intraperitoneal administration of blood products, we estimate that the loss risk is approximately 1%. Thus, the preterm rupture of the membranes 3 weeks after transplantation is likely a consequence of the OI rather than the IUT. If IUT proves to be a realistic alternative, there will be an obvious need for comparative studies between IUT and early postnatal transplantations.

The therapeutic potential of MSC extends to disorders affecting mesenchymal progenitors, such as hypophosphatasia, muscular dystrophia, and mucopolysaccharidosis (*47*). Several of these disorders can be diagnosed prenatally, making them potential candidates for IUT. It is likely that prenatal transplantation in some of these conditions may be more efficacious than postnatal therapy, because the children are already severely affected at birth. In addition, in many of these conditions, the diagnosis is often made rather late during pregnancy, so that termination becomes a cumbersome and often impossible alternative. For these patients, a therapeutic prenatal option would be of great value.

CONCLUSION

Research on MSC is in its infancy, and the clinical indications remain to be established. Our data show that allo-

geneic fetal MSC engraft and differentiate even when the recipient is immunocompetent and HLA-incompatible.

ACKNOWLEDGMENTS

The authors thank Olle Olerup, M.D., for the DRB1 donor-specific primers and Rachel Sugars for assistance with bone immunohistochemistry.

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