Endometrial Cells Derived From Donor Stem Cells in Bone Marrow Transplant Recipients

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Disorders of the uterine endometrium are common, leading to abnormal uterine bleeding, infertility, pregnancy complications, miscarriage, endometriosis, and cancer. Although the generation and growth of the uterine endometrium is important in normal pregnancy and disease, it is poorly understood. The uterine endometrium is one of the most dynamic human tissues and consists of a glandular epithelium and stroma that are completely renewed in each monthly menstrual cycle. Endometrial stem cells are thought to reside in the basalis layer and serve as a source of cells that differentiate to form the endometrium. These endogenous stem cells allow the rapid regeneration of the endometrium necessary to support pregnancy.

Stem cells of bone marrow origin have the ability to transdifferentiate into multiple nonhematopoietic cell lineages. Adult bone marrow stem cells differentiate into hepatocytes, skin, muscle, lung, and neuronal cells in rodents. Human bone marrow-derived circulating stem cells have been shown to take on functions outside the hematopoietic system. Bone marrow–derived circulating stem cells have been shown to take on functions outside the hematopoietic system. These findings demonstrate that endometrial cells can originate from nonuterine stem cells and suggest that nonuterine stem cells contribute to the regeneration of endometrial tissue.

Methods

Study Participants and Endometrial Biopsies

We studied 4 female allogeneic bone marrow transplant recipients who received marrow from a single-antigen mismatched related donor and therefore had an HLA type that allowed determination of the origin of any cell. To avoid the possibility that men may not harbor female reproductive tract stem cells, each recipient had received marrow from female donors. All bone marrow transplants were performed for leukemia treatment. All women were of reproductive age (28-43 years) and had received total body irradiation and chemotherapy with cyclophosphamide and in 2 cases also cytarabine or busulfan at least 2 years before biopsy.

Endometrial biopsies were performed for clinical indications; 1 was performed for the evaluation of infertility after sex steroid treatment and the other 3 for abnormal vaginal bleeding. All endometrial biopsies obtained from HLA-mismatched recipients and identified by the investigator in 1998-2002 were included. An equal number of endometrial biopsies were obtained from healthy nontransplanted control women of reproductive age (32-41 years) as part of fertility evaluation or for abnormal vaginal bleeding. Endometrial biopsies were obtained under an approved human investigation committee protocol. Written informed consent was obtained for clinical indications; 1 was obtained for fertility evaluation or for abnormal vaginal bleeding.
Immunohistochemistry was performed to determine cell identity. Immunohistochemistry revealed endometrial epithelial cells and stromal cells of donor origin in the uterus of women who were bone marrow transplant recipients. Endometrial sections from nontransplanted controls did not demonstrate expression of mRNA for a discrepant HLA type (Figure 1). Because this mRNA may have derived from migratory bone marrow–derived leukocytes, immunohistochemistry was performed to determine cell identity. Immunohistochemistry revealed endometrial epithelial cells and stromal cells of donor origin in the uterus of women who were bone marrow transplant recipients (Figure 2). Endometrial sections from nontransplanted controls did not demonstrate cells with a discordant HLA type. The percentage of bone marrow–derived cells was greater in stroma than glandular epithelium and was localized in focal areas, suggesting local proliferation of donor-derived stem cells. In one patient, the endometrium consisted of more than 50% donor-derived cells (Figure 2C). Others consisted of approximately 0.3%, 4%, and 11% donor-derived cells (Table). Although most glands consisted of entirely host or entirely donor-derived cells, some glands consisted of a fraction of cells of each origin or contained only a few cells of donor origin (Figure 2D, E). Large numbers of transient mature leukocytes of donor origin were observed. Some epithelial cells of donor origin demonstrated cilia characteristic of functionally differentiated secretory endometrium (Figure 2E).

To distinguish CD45-positive leukocytes from endometrial cells, serial immunohistochemistry was performed.
formed on each slide. CD45 is present on all human cells of hematopoietic origin, except erythroid cells and platelets. Identification of leukocytes with CD45 antibody demonstrated the existence of endometrial cells that were not CD45 positive (Figure 3), which confirmed that the immunohistochemical staining was identifying endometrial cells of donor origin rather than migratory mature bone marrow–derived cells.

Immunofluorescence was used to co-localize the discrepant HLA type with calcitonin, which identifies endometrial epithelial cells in the secretory phase of the menstrual cycle. Colocalization of the HLA-discrepant epithelial cells and calcitonin expression demonstrated that cells of donor origin expressed this endometrial cell-type specific marker and were capable of expressing a marker of functional differentiation (Figure 4). Staining with 4,6-diamidino-2-phenylindole, which forms fluorescent complexes with natural double-stranded DNA, did not reveal any evidence of double signal intensity (data not shown), indicating a low likelihood of cell fusion.

COMMENT

In human or murine bone marrow transplant recipients, donor-derived cells can differentiate into numerous cell types of mesodermal, ectodermal, and endodermal origin. This preliminary study suggests that bone marrow–derived cells can differentiate into human uterine endometrium.

Table. Bone Marrow Transplant Recipients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Conditioning Regimen</th>
<th>Time From Transplantation to Biopsy, mo</th>
<th>Positive Cells/Total No. of Cells (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>TBI, cyclophosphamide, cytarabine</td>
<td>157</td>
<td>4950/10 400 (48)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>Cyclophosphamide, busulfan</td>
<td>129</td>
<td>1203/12 300 (10)</td>
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<tr>
<td>3</td>
<td>32</td>
<td>TBI, cyclophosphamide</td>
<td>35</td>
<td>684/17 300 (4)</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>TBI, cyclophosphamide</td>
<td>24</td>
<td>45/19 500 (0.2)</td>
</tr>
</tbody>
</table>

Abbreviation: TBI, total body irradiation.

Figure 2. Donor-Derived Cells in the Endometrium of an HLA-A11–Mismatched Bone Marrow Transplant Recipient (Patient 1)

Immunohistochemistry using anti–HLA-A11 monoclonal antibody, biotin peroxidase detection system, diaminobenzidine as the chromogen (brown), and hematoxylin counterstain. A, HLA-A11–negative control (original magnification ×200). B, HLA-A11 immunopositivity (brown) in an HLA-A11–positive control (original magnification ×200). C, HLA-A11 immunopositivity (brown) in patient 1. More than 50% of the cells were of donor origin (original magnification ×100). D, Endometrial glands partially derived from cells of donor origin (brown; original magnification ×400). E, Rare cells of donor origin (brown) in an endometrial gland. Functional differentiation is noted by characteristic cilia. Arrowheads identify the ciliated epithelial surface (original magnification ×600).

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fractions of donor-derived cells or only a small number of individual cells of donor origin, which implies that not all glands are clonal in origin. The greater number of donor-derived stromal cells compared with glandular epithelial cells suggests differential cell-type–specific rates of transdifferentiation.

The considerable variation in the percentage of cells of donor origin likely depends on the length of time from treatment to biopsy, as well as multiple factors that reflect tissue damage, loss of endogenous stem cells, and tissue repair by recruitment of bone marrow–derived cells; here, these factors include chemotherapy regimen, radiation dosage, graft-vs-host disease, and other endometrial conditions unrelated to the transplant. The woman whose endometrial sample demonstrated the most bone marrow–derived cells had received dual-agent chemotherapy and total body irradiation.

Endometrial ablative techniques in women are fraught with a high long-term failure rate and rarely result in complete long-term absence of endometrial tissue or abnormal endometrial bleeding; this treatment failure occurs despite apparent initial destruction or removal of all endometrial tissue. Conversely, repair of a damaged uterine cavity with little remaining endo-

Figure 3. Donor-Derived Endometrial Cells and Differentiation From Transient Leukocytes

Immunohistochemistry for HLA-A11 using anti–HLA-A11 monoclonal antibody, biotin peroxidase detection system, and diaminobenzidine as the chromogen (brown), and for leukocyte common antigen (CD45) using anti-CD45 monoclonal antibody, biotin peroxidase detection system, and Nova red as the chromogen (red). Hematoxylin was used as the counterstain. A, Control showing concordant HLA-A11–positive epithelial cells (brown) and CD45–positive leukocytes (brown and red) in a nontransplanted individual (original magnification ×200). B, Endometrial glands of HLA-A11–positive donor origin (brown) in HLA-A11–negative transplant recipient (patient 1) and surrounding leukocytes (red). C, Endometrial stromal cells in HLA-A11–negative transplant recipient (patient 1) showing endogenous stromal cells (HLA-A11 negative/CD45 negative; blue [small arrow]); stromal cells of HLA-A11–positive donor origin (HLA-A11 positive/CD45 negative; brown [black arrowhead]); and leukocytes of donor origin (HLA-A11 positive/CD45 positive; brown and red [white arrowhead]) (original magnification ×400).

Figure 4. Differentiation Shown by Immunofluorescence in Donor-Derived Cell in Endometrial Epithelial Layer

Immunofluorescence using tetramethylrhodamine isothiocyanate for calcitonin expression (red) and fluorescein isothiocyanate for donor-derived cells (green) in endometrial epithelial cells of HLA-A11–negative transplant recipient (patient 1) who received bone marrow from an HLA-A11–positive donor. A, Calcitonin expression in endometrial epithelium indicative of receptivity to blastocyst implantation. B, Mismatched HLA-A11–positive endometrial epithelial cell of donor origin (arrowheads). C, Merge demonstrating HLA-mismatched cell expressing calcitonin as a marker of functional differentiation (original magnification ×100).
ENDOMETRIAL CELLS FROM DONOR STEM CELLS

metrium (Asherman syndrome) has a high success rate. Both of these clinical observations suggest that bone marrow–derived regeneration of endometrium has clinical significance.

Ectopic location of the endometrium is characteristic of endometriosis. Endometriosis is a common disease, occurring in approximately 15% of women, causing infertility and pelvic pain. The predominant theory for the origin of endometriosis is retrograde menstruation through the fallopian tubes, with ectopic implantation; however, this theory cannot explain foci of endometriosis outside the peritoneal cavity. A nonendometrial circulating source of stem cells that can result in endometrial cells suggests an alternative origin of some endometriosis. In some instances, endometriosis may arise by differentiation of bone marrow–derived cells into endometrium in ectopic locations. There is an association between endometriosis and immune disorders, perhaps indicating that a single disorder may be common to ectopic bone marrow–derived transdifferentiation of endometrium and other immune phenomena. This hypothesis warrants further investigation.

These preliminary findings suggest that bone marrow–derived cells can generate endometrium, which may have clinical implications for establishing and maintaining pregnancy, treating uterine disorders, and therapeutically augmenting stem cell transdifferentiation into endometrium.

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REFERENCES