Brief report

Little evidence of donor-derived epithelial cells in early digestive acute graft-versus-host disease

Véronique Meignin, Jean Soulier, Frédéric Brau, Marc Lemann, Eliane Gluckman, Anne Janin, and Gérard Socié

Donor origin of epithelial intestinal cells has been studied in animals and humans after transplantation and has been used as evidence of hematopoietic stem cell (HSC) plasticity. However, in the human gastrointestinal tract, no study used X- or Y-chromosome detection by fluorescence in situ hybridization (FISH) coupled with immunologic stainings to characterize cell types on the same tissue section. Here, we combined these techniques on the same section of duodenal epithelium in 6 patients with acute graft-versus-host disease. Donor-derived lymphoid cells were detected in the epithelium and the lamina propria, as expected. However, using our stringent criteria, no donor-derived cells could be proven to be epithelial. (Blood. 2004;103:360-362)

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Study design

The 6 female patients underwent allogeneic, non-T-cell-depleted bone marrow transplantation from a male sibling donor. All received grafts for leukemia, were full donor chimera, and had not experienced relapse at the time of biopsy. Duodenal biopsies were performed for diagnostic purposes during endoscopic examination 14 to 33 days after transplantation because of diarrhea. Biopsy samples from female patients grafted with a male donor were considered positive controls. Female donor were considered negative controls, and those from male recipients grafted with a female donor were considered positive controls.

All patients gave their consent to this study, conducted in accordance with institutional procedures. Approval was obtained from the Hôpital Saint Louis institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

All biopsy samples were fixed in alcohol, formalin, acetic acid (AFA) and were further processed for paraffin embedding. Sister sections 5-µm thick were made on each block; the first section was stained with hematoxylin-eosin. Histologic diagnosis and grading of digestive graft-versus-host disease (GVHD) (Table 1) were established according to criteria previously reported.18 The sister section described here was used for combined FISH, immunohistochemistry, and immunofluorescence methods. For the X and Y FISH procedures, we performed each step of the protocol as described by Johnson et al,19 but we included immunohistochemistry and immunofluorescence stainings. First, we removed the paraffin with xylene and rehydrated the samples with alcohol. Next we performed lymphoid (CD45) immunoperoxidase staining before enzymatic digestion and followed this with hematoxylin counterstaining. At the end of the FISH process, we performed epithelial (AE1/AE3) fluorescence staining.

Reagents were CEP X (Spectrum green)/Y (Spectrum orange) DNA probe (Vysis, Downers Grove, IL), mouse monoclonal anti-CD45 antibody (clones PD7/26 and 2B11; DAKO, Carpinteria, CA), and mouse monoclonal anticytokeratin primary antibody (clone AE1/AE3; Boehringer Mannheim, Indianapolis, IN) revealed by a second AMCA-conjugated horse antimouse immunoglobulin G (IgG) antibody (Vector Laboratories) whose spectrum is the same as that of DAPI. The slides were not counterstained by DAPI but were coverslipped with mounting medium.

Tissue sections were analyzed in a blinded fashion by 2 specialists in pathology and hematology (V.M., J.S.) with an upright epifluorescence microscope (Leica DMR(D), Rueil-Malmaison, France) equipped with 3 appropriate filter blocks for fluorescence analysis (Spectrum green, Spectrum orange, and AMCA) and with bright light. The microscopic pictures were captured through a Plan Apo 63 × 1.32 N.A. oil immersion objective (0.132 mm/pixel; Leica) with a color tri–charge-coupled device camera (3 × 1/2") LEI-750D CE system and were recorded on a personal computer using a Matrox Meteor PCI frame grabber board (Leica) and Leica Q-Winswefware.

The number of X signals in the epithelium and the lamina propria and the number of Y signals in the lamina propria were counted on 500 cells. The number of Y signals in the epithelium was counted on 5000 epithelial cells (Table 1). Whenever we detected a Y signal in a cell localized in the...
epithelium, 4 pictures, 1 in each of the 3 fluorescence spectrums and 1 in bright light, were captured. Microscopic pictures were matched with the GIMP software (http://www.gimp.org) to eliminate nonspecific signals and to analyze the types of chimeric cells.

### Results and discussion

In positive controls (male recipients from male donors), X and Y signals were, respectively, 81 versus 73 per 100 cells in the lamina propria and 75 versus 82 per 100 cells in the epithelium. In negative controls (female recipients from female donors), we did not find any Y-chromosome–specific signals. In female patients who received grafts from male donors, Y-chromosome–specific signals were more numerous (3-36 [mean, 13.2] signals per 100 cells) in the lamina propria compared with the epithelium (0.00-0.16 [mean, 0.053] signals per 100 cells) (Table 1). By matching microscopic pictures, we demonstrated that 60% to 83% (mean, 69.1%) of the signals in the lamina propria and 50% to 100% (mean, 91.6%) of the signals in the epithelium were of hematopoietic origin because they were CD45 labeled (Figure 1A). These mononuclear cells probably were lymphocytes or macrophages infiltrating the digestive tract. In the lamina propria, the non–CD45-labeled cells could be stromal cells or even myofibroblasts, as previously reported. In the epithelium, only 2 donor-derived, CD45− XY cells (0.04 per 100 cells) were found in a single patient. They were not of hematopoietic origin because they did not express the CD45 marker. However, no epithelial staining was detected in these cells, thus raising questions about their nature (Figure 1B).

Our results differ from those previously published regarding the human digestive tract after HSC transplantation. Two previous studies10,11 suggested that though epithelial cells of donor origin were not numerous (0.4%-3.6% for Okamoto et al11 and 4%-6% for Körbling et al10), they were regularly found. In addition, these studies were performed in sex-mismatched transplantations, but technical conditions used for our tissue study were different. We used the XY FISH method, which allows for the elimination of most nonspecific, large-spectrum fluorescence. We also combined FISH and immunostaining results and analyzed them on the same tissue section to avoid serial consecutive sections, which favors localization bias. In Körbling et al,10 successive sections were used.

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### Table 1. FISH and immunostaining results

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<tr>
<th>Lamina propria</th>
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FISH signals were counted as follows: X signals on 500 cells in epithelium and lamina propria; Y signals on 500 cells in lamina propria and on 5000 cells in epithelium. All results included in this table are related to 100 cells. BMT indicates bone marrow transplantation; —, not applicable.

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Figure 1. FISH and immunostaining results on identical duodenal sections of 2 female patients who underwent allogeneic, non–T-cell–depleted bone marrow transplantation from male sibling donors. The epithelial basal membrane appears with a broken line in all the images. (A) In the epithelium, a cell with a red signal (white arrow) corresponds to Y-chromosome labeling (FISH; original magnification, ×630). X chromosomes are stained green. (Al) The same cell (white arrow) is not stained with the anticytokeratin antibody (indirect immunofluorescence; original magnification, ×630). Epithelial cells are stained white on a blue background (open black arrowhead). (Alii) The same cell (white arrow) is stained with the anti–CD45 antibody (indirect immunoperoxidase; original magnification, ×630). A lymphoid cell is stained brown in the lamina propria (white arrowhead). (Aiii) Higher magnification of panel Ai centered on the XY cell in the epithelium (FISH; original magnification, ×3150). X chromosome, green; Y chromosome, red. (B) In the epithelium, a cell with a red signal (white arrow) corresponds to Y-chromosome labeling (FISH; original magnification, ×630). X chromosomes are stained green. (Bi) The same cell (white arrow) is not stained with the anticytokeratin antibody (indirect immunofluorescence; original magnification, ×630). (Bii) The same cell (white arrow) is stained with the anti–CD45 antibody (indirect immunoperoxidase; original magnification, ×630). (Biii) Higher magnification of panel Bi centered on the XY cell in the epithelium (FISH; original magnification, ×3150). X chromosome, green; Y chromosome, red.
whereas in Okamoto et al,\textsuperscript{11} Y-chromosome staining alone was used (not dual XY staining). Finally, during early acute GVHD of the digestive tract, little evidence of donor-derived epithelial cells was found in the patient studied by Körbling et al\textsuperscript{10} (6% on day 60) and the 2 patients studied by Okamoto et al\textsuperscript{11} (0.4% and 0.6% on day 26). Thus, in addition to technical difficulties that could be overcome with the method we used, there are few signs of donor-derived epithelial cells early after HSC transplantation. On the other hand, the 2 male cells we found within the duodenal epithelium may indeed represent the first step in cell fusion, a mechanism that seems to be responsible for bone-marrow–derived stem cell repair of damaged tissue.\textsuperscript{21}

References

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