Breast Implant Capsules Are Partially Composed of Bone Marrow–Derived Cells

Casey Isom, MD, Vishal Kapoor, MD,* Lynne Wilson, BA,* Carrie Fathke, MD,* Leigh Barnes, BA,* Stephen R. Sullivan, MD,* and F. Frank Isik, MD†*

Abstract: Capsular contracture is the most common complication following breast augmentation or reconstruction with implants. We recently demonstrated that bone marrow–derived cells provide fibroblasts to murine skin during wound healing. To determine if bone marrow–derived cells were the cellular source of periprosthetic capsules, we created chimeric C57BL mice containing bone marrow cells from isogeneic enhanced green fluorescent protein ($EGFP^+$) mice and implanted with a textured silicone shell implant. We found that none of the mice developed infection or capsular contracture, but day 30 capsules were composed of 26.4 ± 6.1% $EGFP^+$ cells, and day 60 capsules had 21.8 ± 10.3% $EGFP^+$ cells. Immunohistochemistry revealed a small population of $EGFP^+$ cells in the capsules that were myofibroblasts. Thus, breast implant capsules are partially composed of bone marrow–derived cells and, given the potential of these cells to become myofibroblasts, may explain the cellular source of capsular contracture when it develops.

Key Words: capsular contracture, bone marrow, mesenchymal stem cells, breast implant

(*Ann Plast Surg* 2007;58: 377–380)

Capsular contracture is the most common long-term complication following the successful use of prosthetic implants for breast augmentation or breast reconstruction.[1,2] Depending on the severity of the contracture, patients can develop firmness and distortion of the augmented or reconstructed breast, which in severe cases may become painful. Often the best treatment of severe capsular contracture is capsulotomy or capsulectomy, with replacement or removal of the implant, which may lead to patient and physician dissatisfaction.[3]

Insertion of a foreign body will elicit an initial immune response and the development of a synovial-like capsule, which can be of varying thickness and cellularity.[4] Though the etiology of capsular contracture remains multifactorial, evidence is compelling that chronic low-grade infection with the *Staphylococcus epidermidis* group is a likely cause.[5–8] Other etiologies that have been correlated with capsular contracture include prior chest wall radiation, the choice of filler material (silicone versus saline), implant placement (subglandular versus subpectoral), surface texture (smooth versus textured), and inflammation.[1–4,9–14]

Whether caused by low-grade chronic infection or other etiology, the underlying pathophysiology of capsular contracture is excessive contraction of the thickened fibrous capsule. While histologic studies of explanted capsules from both humans and animals have implicated myofibroblasts as the predominant cell type,[15,16] the source of these cells has not been elucidated. Because the implants initially develop an inflammatory reaction, it is likely that inflammatory cells could be the source of the capsule. Given the plasticity of bone marrow–derived cells, it is quite feasible the myofibroblasts originate from the bone marrow.[17] Based on these assumptions and our recent finding that the bone marrow provides a substantial contribution of the "fibroblasts" in the murine skin and during wound healing,[18] we hypothesized that the bone marrow is the cellular source of the capsules and myofibroblasts around breast implants. To track the fate of bone marrow–derived cells in an animal model, we used the chimeric mouse in which the bone marrow from enhanced green fluorescent protein ($EGFP^+$) transgenic mice is transplanted into normal C57BL mice. We present evidence that the bone marrow–derived cells are a source of the cells that form the capsule surrounding breast implants.

MATERIALS AND METHODS

Chimeric Mouse Preparation

All animal procedures are in accordance with the Guide for the Care and Use of Laboratory Animal (National Institutes of Health [NIH] publication No. 86-23) and have been approved by the Animal Care Committee of the University of Washington. For the generation of chimeric mice, bone marrow was collected from the tibia and femur of $EGFP^{+/-}$ (C57BL/6-TgN (ACTbEGFP)10sb) donor mice (Jackson Laboratories, Bar Harbor, ME). A single cell suspension was created and the cells prepared for immediate transplantation. Recipient adult C57BL mice (Jackson Laboratories) were immunodepleted using busulfan (25 mg/kg subcutaneously; Sigma, St. Louis, MO). After 6

Received July 7, 2006, and accepted for publication, after revision, July 29, 2006.

From the *University of Washington Medical Center; and †The Polyclinic, Seattle, WA.

This work was supported by a grant from the NIH: R01 GM073624.

Reprints: Frank Isik, MD, Department of Surgery, Box 356410, University of Washington Medical Center, 1959 NE Pacific Street, Seattle, WA 98195. E-mail: isik@u.washington.edu.

ISSN: 0148-7043/07/5804-0377
DOI: 10.1097/01.sap.0000243996.37786.4a

days of receiving daily busulfan, mice received 10^5 EGFP$^+$ bone marrow cells suspended in PBS via tail vein injection. Two, 3 and 10 weeks posttransplantation, peripheral blood from chimeric mice was analyzed for recovery of the total leukocyte counts. Degree of chimerism was assessed by flow cytometry of circulating nucleated cells at 10 weeks.

Animal Implant Model

After 10 weeks and full recovery of the circulating leukocytes and confirmed chimerism for EGFP >70%, the chimeric mice were anesthetized through intraperitoneal injection of ketamine (15 mg/kg) and xylazine (1 mg/kg; Phoenix Pharmaceuticals Inc, St. Joseph, MO). The dorsal hair was shaved and the skin was prepped; a vertically oriented dorsal incision was made to accommodate a 1.0-cm^2 portion of a textured implant shell made of silicone (Siltex, Mentor Corporation, Santa Barbara, CA). The wound was closed with 4-0 nylon sutures and covered with a transparent semi-occlusive dressing (Tegaderm, 3M, MN).

Seven mice were implanted with a total of 9 silicone shells. Five mice had single implants and 2 mice had 2 implants placed. On postimplant day 30, 3 mice (with 4 implants) were euthanized and the entire implant was excised en bloc with surrounding soft tissue. The remaining 4 mice (with 5 implants) had their implants harvested on postimplant day 60. The implants (consisting of implant, capsule, skin, and subcutaneous tissue) were divided and either fixed in 2% paraformaldehyde for 2 hours and then embedded in O.C.T. (Tissue-Tek, Sakura, Torrance, CA), or placed directly in O.C.T., and frozen. Tissues were then cut on a microtome and mounted for histologic analysis by fluorescent microscopy. Slides were counterstained with DAPI (Boehringer-Mannheim, Indianapolis, IN) for 5 minutes at room temperature to establish tissue architecture. Expression of EGFP in tissues was evaluated by fluorescence microscopy using a wide-band FITC filter on 10-μm tissue sections. Using immunohistochemistry, sections were stained with PE-conjugated anti–heavy-chain myosin antibodies to determine presence of myofibroblasts. Corresponding sections of normal skin (noncapsule) were similarly processed as an internal control for baseline levels of EGFP$^+$ cells in the native skin. Manual cell counts of 5 random tissue sections were used to determine the percentage of EGFP$^+$ cells within the capsules. Counts are expressed as mean ± SD. Slide images were digitized using IP Laboratory Spectrum (Scanalytics, Fairfax, VA) and subsequently processed in Photoshop Version 8.0 (Adobe Systems Incorporated, San Jose, CA).

RESULTS

Generation of EGFP Chimeric Mouse

All 7 mice survived chemotherapy for bone marrow stem cell ablation and stem cell rescue, with good recovery of the circulating leukocytes after 10 weeks (Fig. 1). All mice achieved good reconstitution of their bone marrow with EGFP$^+$ cells, on average 76% (72%–85%). No animal showed signs of graft-versus-host disease or immunologic signs of bone marrow rejection.

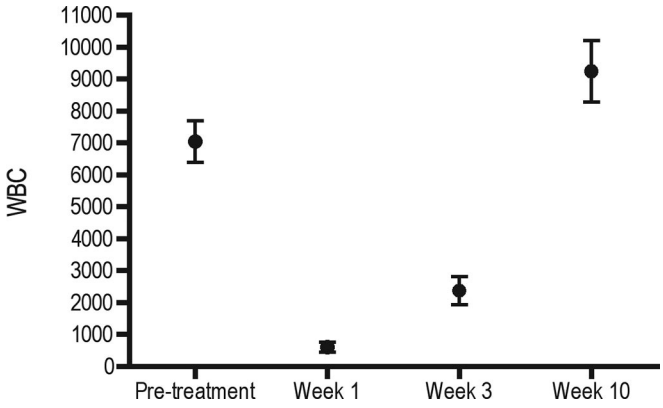

FIGURE 1. White blood cell count during busulfan treatment and following bone marrow transplant, showing good recovery of circulating white blood cells.

Analysis of Capsules

All mice healed their implant incisions, and there were no cases of implant infection or implant extrusion. None of the mice developed capsular contracture based on manual compression of the implant as the implant shells and surrounding capsules remained soft. Day-30 capsules formed in the hypodermis above the panniculus carnosus muscle and were partially composed of EGFP$^+$ cells (Fig. 2A and B). Manual counts of the day-30 capsules showed that 26.4 ± 6.1% of the cells were EGFP$^+$. When compared with the dermis and epidermis above, the capsules harbored visibly greater numbers of EGFP$^+$ cells. From our prior work with this animal model, the dermis and epidermis contains 9%–15% EGFP$^+$ cells.

Day-60 capsules also formed around the implants in the hypodermis above the panniculus carnosus muscle. Capsules at day 60 also had greater numbers of EGFP$^+$ cells than the more superficial layers of the skin, but the overall contribution of bone marrow–derived cells was less than in the day-30 capsules (21.8 ± 10.3% EGFP$^+$). This observation suggests that the cellular contribution from the bone marrow decreased with time. There were areas of both day-30 and day-60 capsules that had very few or no EGFP$^+$ cells.

Analysis of the tissues using immunohistochemistry revealed EGFP$^+$ cells within the capsules and some EGFP$^+$ cells that contained heavy-chain myosin (Fig. 2C), a marker used to identify myofibroblasts. Only few of the myofibroblasts were not EGFP$^+$. This result indicates that the myofibroblasts within the capsule can be bone marrow–derived.

DISCUSSION

In this study, we found that bone marrow–derived cells integrated in the periprosthetic capsules. The contribution from the bone marrow decreased with time but still comprised about one quarter of the pericapsular cell population. These data suggest that in the absence of infection, the cellular source of the capsule is three quarters from the local subcutaneous cell population and one quarter from circulating bone marrow–derived cells. This insight may have implications for the treatment of capsular contracture.

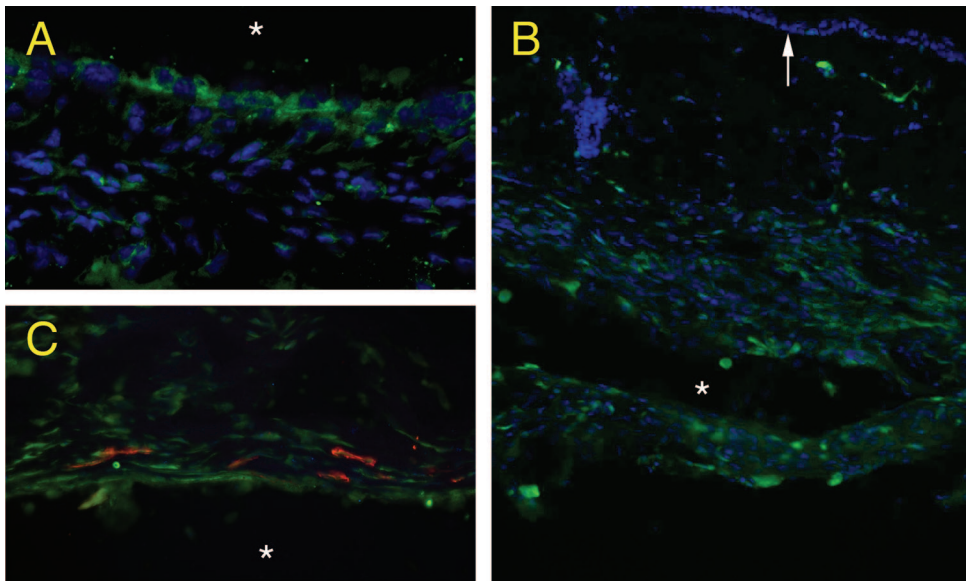

FIGURE 2. A, Day-30 implant capsule with EGFP$^+$ cells congregating mainly along the inner surface of the capsule next to the implant (implant space designated by *). Blue represents DAPI-stained nuclei. B, Lower-power view of a 30-day specimen showing the overlying skin (white arrow) and the implant space (*). Note the enriched EGFP$^+$ cell population around the implant in comparison with the adjacent overlying skin. C, Day-30 implant capsule (implant space shown at bottom of figure with *) with bone marrow–derived EGFP$^+$ cells lining the capsule (green cells). Staining of tissue sections with anti–heavy-chain myosin shows the presence of myofibroblasts (red staining) adjacent to the capsule and colocalizing with the EGFP$^+$ cells, confirming the origin of some of the myofibroblasts as bone marrow–derived cells.

The development of significant capsular contracture remains the most common and adverse outcome of breast augmentation or reconstruction with prosthetic implants. It is not clear why some implants develop capsular contracture, although there are various theories such as infection, hematoma, and an autoimmune response. Furthermore, it may occur months or even years after implantation.[19] A large volume of data that has been amassed over the years seems to suggest that infection is central to the development, although clinically overt infections are rare and on the order of 1%–3%. One potential hypothesis to explain capsular contracture is that a subclinical infection triggers a greater bone marrow response with the influx of inflammatory cell types to combat the infection. Since our mice did not develop an infection or capsular contracture, we cannot confirm this hypothesis. However, we suspect that in the setting of a chronic subclinical infection as seen with *S. epidermidis* involvement, the percentage of bone marrow–derived cells and myofibroblasts would increase.

In summary, our data show that approximately one quarter of the cells that compose the periprosthetic breast implant capsules are derived from the bone marrow. Immunohistochemistry revealed that some capsular EGFP$^+$ cells are indeed myofibroblasts. Although we could not confirm with our present study, we speculate that bone marrow–derived cells are a prominent contributor to capsular contraction in the setting of increased inflammation, as seen after radiation treatment or a chronic subclinical infection.

ACKNOWLEDGMENTS

The authors acknowledge the generous support from National Institutes of General Medical Sciences and NIH R01 GM073624.

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