Myelointegration of Titanium Implants: B Lymphopoiesis and Hemopoietic Cell Proliferation in Mouse Bone Marrow Exposed to Titanium Implants

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Multinucleated giant cells have been observed at interfaces between bone marrow and titanium implants in mouse femurs. This raises concern that macrophage-derived factors might perturb local lymphohemopoiesis, possibly even predisposing to neoplasia in the B lymphocyte lineage. It has been found that an implant-marrow interface with associated giant cells persists for at least 1.5 years. Precursor B cells show early increases in number and proliferative activity. At later intervals, however, they do not differ significantly from controls, and there are no perturbations in spatial localization of either B lineage cells or DNA-synthesizing hemopoietic cells. The results of this investigation in mice demonstrate that, following initial marrow regeneration and fluctuating precursor B cell activity, and despite the presence of giant cells, titanium implants apparently become well-tolerated by directly apposed bone marrow cells in a lasting state of "myelointegration." (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:175–184)

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Titanium implants as used in both clinical dentistry and orthopedics become intimately associated with bone, a process whose end result has been termed *osseointegration*.[1–3] Previous work using miniaturized titanium implants placed into the femoral diaphysis provided the first demonstration of osseointegration in mice.[4] In addition, an extensive surface of the implant was found to maintain contact with regenerated bone marrow for prolonged periods after surgery. At the implant-marrow interface, macrophage-like cells and multinucleated giant cells develop,[4] suggesting the induction of a local foreign body response. This raises the possibility that factors secreted by activated macrophages and giant cells at the implant-marrow interface might perturb local blood cell production. Of particular concern would be long-term effects on B lymphopoiesis that might carry the risk of initiating B cell abnormalities, including neoplasia.[5]

The bone marrow is the site of hemopoiesis and B lymphopoiesis, controlled within a highly organized microenvironment by local and long-range regulatory factors.[6–10] Large numbers of B lymphocytes are continuously produced in the bone marrow and migrate through the bloodstream to peripheral lymphoid tissues to maintain humoral immunity.[7] During their differentiation in bone marrow, proliferating precursor B cells pass through a series of phenotypically distinct stages,[11,12] during which individual gene segments become combined to produce functional immunoglobulin (Ig) genes encoding B cell antigen receptors.[13–16] This process of DNA recombination is subject to a variety of errors, including chromosomal translocation.[17] Certain

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human B cell leukemias and lymphomas appear to have been initiated in precursor B cells during Ig gene recombination in bone marrow.[17–19] While an initial genetic error may thus be sustained by precursor B cells, a long latent period may precede the appearance of an oligoclonal or monoclonal tumor. During this period, the cells sustain other genetic errors that collectively are required to confer sustained proliferation and cell survival.

The risk of B cell oncogenesis appears to be increased by factors that increase the number and proliferative activity of precursor B cells. Mice carrying a c-myc transgene in the B cell lineage or a transgene for the B cell cytokine interleukin-7 develop B cell lineage tumors, preceded by a lengthy prelymphomatous period of B cell hyperplasia in the marrow.[20,21] A similar effect appears to be produced by protracted macrophage activation. Circulating secretory products from activated macrophages in the spleen stimulates precursor B cell proliferation in bone marrow.[22–24] Chronic granulomata, rich in activated macrophages, produce B cell tumors in certain mouse strains, preceded by sustained stimulation of early precursor B cells in bone marrow.[25,26] Thus the presence of activated macrophages within the bone marrow itself, even if restricted to only a small region of bone marrow, raises the concern that the incidence of B cell neoplasms might be enhanced after a latent period. Since many clinical implants in bone must necessarily transgress the medullary cavity, it becomes important to determine whether the presence of a titanium interface and associated macrophage activity might be associated with changes in the number and proliferative activity of precursor B cells and other hemopoietic cells. This is especially important in the peripheral regions of the bone marrow cavity near the endosteal bone-lining layer, where early precursor B cells and primitive hemopoietic progenitor cells are most concentrated.[5,6,27,28]

While the process of osseointegration is concerned with achieving molecular acceptance and mechanical stability of titanium implants in bone, the term *myelointegration* has been introduced to describe the biocompatibility of implanted materials with bone marrow.[5] Successful myelointegration is an important criterion in determining host tolerance to biomaterials. The purpose of the present study has been to determine whether the presence of a titanium implant impinging for prolonged periods on the medullary cavity of a long bone in mice may exert adverse local effects on the bone marrow, as evaluated by the criteria of B lymphopoiesis and hemopoietic cell proliferation. The frequency and mitotic activity of B lineage cells in the bone marrow of mouse femora housing titanium implants has been examined using the B220 cell surface glycoprotein as a marker for the B cell lineage.[29] Subpopulations of precursor B cells in bone marrow cell suspensions were defined by labeling intracytoplasmic and cell surface μ heavy chains of IgM (cμ and sμ) and the intranuclear enzyme terminal deoxynucleotidyl transferase (TdT), which is expressed only during stages at which μ heavy chain genes are undergoing rearrangement.[11,30,31] Although some transient disturbances have been observed, the results are reassuring in showing no overall effect on the number and proliferative status of B lineage cells over long periods. In addition, in vivo radiolabeling and radioautography of bone marrow sections showed no long-term alterations in the spatial distribution of either B lineage cells or total DNA synthesizing cells at the bone marrow-titanium interface.

MATERIALS AND METHODS

Mice

Male C3H/HeJ mice (Charles River, St-Constant, Quebec, Canada) were fed Purina Lab Chow ad libidum and used at 4 weeks of age (15 to 20 g body weight).

Implant Preparation

Implants (1.0 mm long, 0.58 mm wide) made of commercially pure titanium were produced at the Institute of Applied Biotechnology, Göteborg, Sweden, as previously described and illustrated.[4] The implants were handled only by titanium-coated instruments. Implants were cleaned and sterilized, as described.[4]

Surgical Procedure and Implant Placement

The surgical procedure of placing a titanium implant across 1 femur of a mouse was performed as previously described.[4] Briefly, mice were anesthetized with an intraperitoneal injection of 1.25% Avertin and placed in a laminar flow hood to minimize the risk of infection. A skin incision was made along the lateral aspect of the thigh, the muscles of the thigh were separated, a small hole was gently drilled through the cortex of the mid-diaphysis of the femur under saline irrigation, and an implant was placed. As previously described, the implant had a grooved shaft that would become anchored into the cortical bone and a smooth tip that entered the medullary cavity, containing the adjacent bone marrow.[4] The skin was sutured and the mice were monitored daily. Sham-operated mice, in which a hole was drilled in 1 femur without placing a titanium implant, were used as controls.

Cell Preparation

Mice were sacrificed by cervical dislocation. Femurs were dissected and bone marrow cells were flushed out and suspended in cold 10% (v/v) newborn calf serum (NCS) in minimal essential medium (MEM) (Gibco, Grand Island, NY). Cell suspensions were washed through NCS by centrifugation, nucleated cells were enumerated by an electronic particle counter (Coulter Electronics, model B, Hialeah, CA), and cell concentrations were adjusted to 40.0 $\times$ 10^6 cells/mL of MEM/NCS.[29,32]

Antibodies and Radioiodination

Purified rat anti-mouse B220 monoclonal antibody (mAb) 14.8, concentrated (2 mg/mL) from hybridoma cell culture supernatants (American Type Culture Collection, Rockville, MD), was used at 1:150 dilution.[29] Fluorescein- (FITC) goat anti-rat IgG (1:20 dilution; Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used as a secondary antibody to detect mAb 14.8. An affinity-purified rabbit anti-TdT antibody (1:20 dilution; Supertech Inc, Bethesda, MD) was detected by rhodamine- (TRITC) conjugated–goat anti-rabbit IgG $F(ab')^2$ (1:20 dilution; Jackson Immunoresearch Laboratories, Mississauga, Ontario, Canada). FITC- and TRITC-goat anti-mouse μ (1:10 dilution, Kirkegaard & Perry; and 1:20 dilution, Southern Biotechnology Assoc, Birmingham, AL) were used to bind μ heavy chains of both surface immunoglobulin (sμ) and cytoplasmic μ (cμ). All antibodies were ultracentrifuged before use (120,000 $\times$ g; 30 minutes) to remove any aggregates.

Monoclonal antibody 14.8 was radioiodinated using a modification of the chloramine-T method.[33] Briefly, purified mAb 14.8 (125 μg) was coupled to carrier-free Na ^{125}I (2mCi, specific activity 1.5 $\times$ 10^7 μCi/μg), resulting in a 50 μg/mL mAb concentration.

Immunofluorescence Staining and Mitotic Arrest

Mice were injected with vincristine sulfate intraperitoneally (Eli Lilly, Toronto, Ontario; l mg/kg body wt) to arrest cells in metaphase. Bone marrow cells were examined 2 hours 40 minutes later, a time interval when the fraction of cells in metaphase provides a sensitive index of the rate of entry into mitosis.[32] Bone marrow B lineage cells were then immunolabeled, as described.[29,32] Briefly, for double labeling of sμ and cμ, cell suspensions from individual femurs were first incubated with FITC-anti-mouse μ (0°C, 30 minutes) to label mature $sμ^+$B cells; washed by centrifugation (200 $\times$ g, 3 minutes, 4°C); deposited onto glass slides in a cytocentrifuge (1100 rpm, 5 minutes; Shandon Southern Instruments, Selwicky, PA); fixed with cold 5% glacial acetic acid in ethanol (0°C, 12 minutes; and washed in phosphate-buffered saline (PBS, pH 7.2). The fixed cytocentrifuged cells were then exposed to TRITC-anti mouse μ (30 minutes at room temperature) in a humidified chamber to label $cμ^+$ precursor B (pre-B) cells and washed in PBS.

For double labeling of B220 and total μ chains (cμ and sμ), cell suspensions were exposed to mAb 14.8 (0°C, 30 minutes); washed; incubated with FITC-anti-rat IgG; washed; cytocentrifuged; fixed; and then incubated with TRITC–anti-mouse μ, as described above. For double labeling of B220 and intranuclear TdT, bone marrow suspensions were exposed to mAb 14.8 and FITC-anti rat IgG, as described above, cytocentrifuged, fixed in 100% methanol (30 minutes at 0°C), and washed in PBS. The fixed, cytocentrifuged cells were then exposed to rabbit anti-TdT (overnight at room temperature), washed in cold PBS, incubated with TRITC-goat anti-rabbit IgG $F(ab')^2$ (30 minutes at room temperature) in a humidified chamber, and washed again. All cytospots were finally washed in PBS overnight to reduce potential background and mounted in a glycerol medium containing p-phenylenediamine to reduce fading of the fluorescent signal.[32]

Immunofluorescence Analysis

Labeled cells were detected by epifluorescence microscopy and cells in metaphase by phase contrast microscopy ($\times$100 oil objective). Cells were scored as either single labeling with FITC alone ($sμ^+$ or $B220^+$) and TRITC alone ($cμ^+$ or TdT^+), or double labeling with FITC plus TRITC ($sμ^+cμ^+$; TdT^+ $B220^+$). At least 2,000 to 4,000 nucleated cells were counted in each case to determine the incidence of labeled cells. The absolute number of labeled cells for each phenotype was determined by multiplying their percentage incidence by the total number of nucleated cells per femur.

In Vivo Labeling of B Lineage Cells and Dividing Cells

To label B lineage cells in situ, 3 mice were anesthetized with an intraperitoneal injection of 1.25% Avertin. ^{125}I-mAb 14.8 (100 μL) was injected into the jugular vein and allowed to circulate for 3 minutes. To rinse out any unbound antibodies, mice were then perfused through the heart with Ringer's Lactate solution and finally perfusion-fixed with a cocadylate-buffered solution (pH 7.2) of 2.5% glutaraldehyde and 2% acrolein for 10 minutes.[4,28]

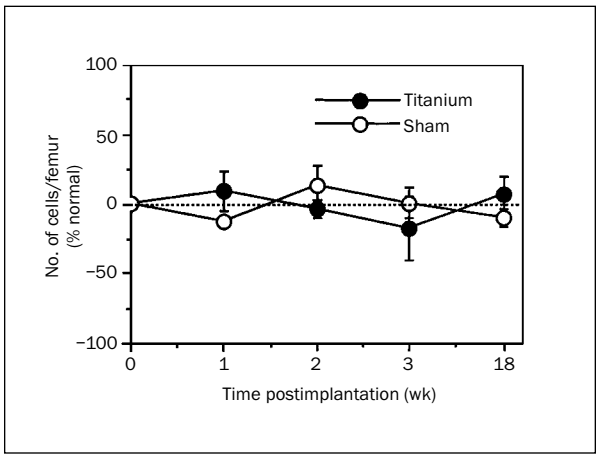

Fig 1 The total number of nucleated cells in the bone marrow of mouse femoral shafts following either the surgical placement of titanium implants or a sham operation. Values are expressed as the number of cells per femur relative to the corresponding number in untreated contralateral femora, represented by the zero reference line. Each point is based on groups of 3 to 4 mice.

To label dividing cells in situ, 3 mice were injected intraperitoneally with tritiated thymidine (^{3}H-TdR), 10 μCi/gm body weight, and sacrificed 1 hour later by perfusion fixation, as above.

Tissue Processing and Radioautography

Femora were decalcified in 10% EDTA (pH 7.2) for 2 weeks, postfixed in 1% potassium ferrocyanide-reduced osmium tetroxide, dehydrated in acetone, and embedded in Epon resin. Titanium implants were removed by a fracture technique and re-embedded in resin, and semi-thin (0.5 μm) transverse sections of femoral shaft were made through the implant site, as described.[4,34] Sections were placed on glass slides, stained with alum/iron hematoxylin, dipped in NTB-2 photographic emulsion (Ilford, Eastman Kodak, Rochester, NY) and exposed for 30 to 40 days.[33] Three or four implanted femora were examined at each experimental time interval studied.

Radioautographic Analysis

Light microscopic radioautographs of bone marrow sections were examined using an ocular grid of successive 2025 μm^2 fields at 100× oil objective. The total number of nucleated cells (excluding megakaryocytes, adipocytes, and endothelial cells) and their overlying silver grains were recorded and mapped across the entire section of each femoral shaft.

Statistical Analyses

A 2-way analysis of variance was performed on the values for the absolute numbers of cells per femur.

RESULTS

Cellularity of Bone Marrow in Titanium-Implanted Femora

In experimental mice, a titanium implant was placed into 1 femur, while the untreated contralateral femur provided an internal control. In sham-operated control mice, also, the surgically exposed femur was compared with the contralateral undisturbed femur. The total number of nucleated cells in suspensions of bone marrow from mouse femora bearing a titanium implant was measured at intervals of 1, 2, 3, and 18 weeks postimplantation. Although some relatively low values occurred after 3 weeks, no overall changes in marrow cellularity relative to the respective untreated contralateral femora were observed (Fig 1). Moreover, a 2-factor analysis of variance showed no statistically significant difference (n = 24, $P > .05$) in bone marrow cellularity between experimental and sham-operated mice throughout the 18-week period (Fig 1).

B Lymphocytes and Precursor B Cells in Bone Marrow of Titanium-Implanted Femora

B lineage cells in bone marrow were quantified by double immunofluorescence labeling at 4 phenotypically distinct stages of differentiation: *(1)* B lymphocytes expressing surface IgM molecules; *(2)* their immediate precursors, pre-B cells, expressing cytoplasmic μ chains (cμ) but not surface μ chains (sμ); and *(3)* pro-B cells, the earliest B lineage cells before the synthesis of μ chains, expressing surface B220 but lacking μ chains (B220$^+$μ$^-$), including *(4)* cells undergoing Ig heavy chain gene rearrangement and characterized by TdT expression (Fig 2).

While sham-operated mice showed virtually no changes in the number of B lineage cells in femoral marrow relative to the contralateral femur (Fig 2), transient changes were apparent in the femora bearing titanium implants (Fig 2). The mean number of IgM$^+$ B lymphocytes appeared to decline in titanium-implanted femora, relative to the contralateral femur, from 1 to 3 weeks after implantation. The number of cμ$^+$sμ$^-$ pre-B cells increased by 75% at 1 week after implantation, a significant change compared with sham-operated mice (Student's t test; $P < .05$), then fell progressively to apparently subnormal levels at 3 weeks. The B220$^+$μ$^-$ pro-B cells also appeared to be depressed in numbers at this time. By 18 weeks, however, all the above cell populations had returned to normal. The only apparent aberration was a variable increase in numbers of TdT$^+$ cells (Fig 2). A 2-way analysis of variance revealed no overall effect attributable to the presence of the implant on any of the B lineage cell populations throughout the 18-week period examined (n = 24, $P < .05$).

A measure of the rate of cell production among proliferating precursor B cells ($c\mu^+s\mu^-$; $B220^+\mu^-$, TdT^+) was provided by the number of cells of each phenotype that accumulated in metaphase at a standard time interval (2 hours 40 minutes) after administering vincristine sulfate to induce mitotic arrest.[32] During the first 3 weeks, pro-B cells and pre-B cells in titanium-implanted femora showed variable proliferative activity, not statistically different from sham-operated controls (Fig 3). At 18 weeks, none of the precursor B cell populations showed any enhancement of production rate in the presence of a titanium implant (Fig 3).

In Situ Localization of $B220^+$ Cells and Proliferating Cells at the Bone Marrow–Titanium Interface

To localize B lineage cells in bone marrow exposed to an established titanium implant, mice were administered ^{125}I-mAb 14.8 intravenously at 18 weeks postimplantation. The distribution of labeled $B220^+$ cells around the site of the titanium implant was visualized by light microscope radioautography of bone marrow sections cut transversely through the femoral shaft and implant site after removal of the implant (Fig 4a). The number of cells labeled with numerous overlying silver grains was recorded and mapped in successive microscopic fields throughout the entire cross section of the implant site (Fig 5a).

An extensive bone marrow–titanium interface still persisted at 18 weeks postimplantation (Figs 4 and 5), as reported previously.[4] The bone marrow had regenerated with apparently normal structure and cellular composition up to the titanium surface, with no intervening tissue. The distribution of $B220^+$ cells around titanium implants was similar to that found in equivalent regions of bone marrow sections from contralateral femora. The B lineage cells were distributed throughout the bone marrow, with no distinct regions of accumulation or depletion (Fig 5a). Similar cell distributions were evident in histologic maps from 2 other mice (data not shown).

To examine the possibility of even longer-term effects, dividing cells were localized in femora of mice 1.5 years after placing a titanium implant. ^{3}H-thymidine was injected 1 hour before sacrifice, and labeled DNA-synthesizing cells were detected in radioautographic tissue sections (Fig 4b). An extensive surface of the implant still remained in direct contact with bone marrow (Figs 4b and 5b). Some cells at the interface had the morphology of macrophages and multinucleated giant cells (Fig 4b), as reported previously.[4]

In untreated contralateral femora, labeled DNA-synthesizing cells were detected mostly in the outer zone of bone marrow near the surrounding bone. Only a few labeled cells were found around the central venous sinus (Fig 5b). In titanium-implanted femora, the DNA-synthesizing cells were also situated predominantly near the periphery of the bone marrow. Only a few labeled cells were found near either the titanium interface or the multinucleated giant cells (Fig 5b). Similar maps of dividing cells were obtained from 2 other mice (data not shown).

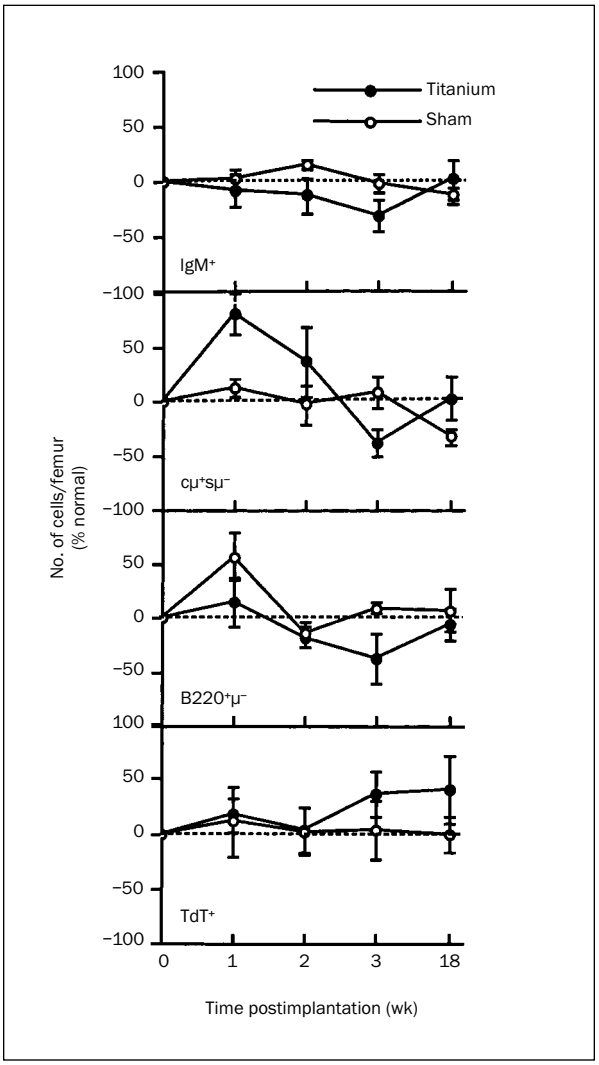

Fig 2 The number of B lymphocytes and their precursors in the bone marrow of mouse femoral shafts following either the surgical placement of titanium implants or a sham operation. Values are expressed as the number of cells per femur relative to that found in respective untreated contralateral femora, represented by the zero reference line. The values were calculated from the incidence of IgM^+ B lymphocytes, $c\mu^+s\mu^-$ pre-B cells, and $B220^+\mu^-$ and TdT^+ pro-B cells, each derived from counts of 2,000 nucleated cells, combined with the total bone marrow nucleated cellularity. Each point is based on groups of 3 to 4 mice.

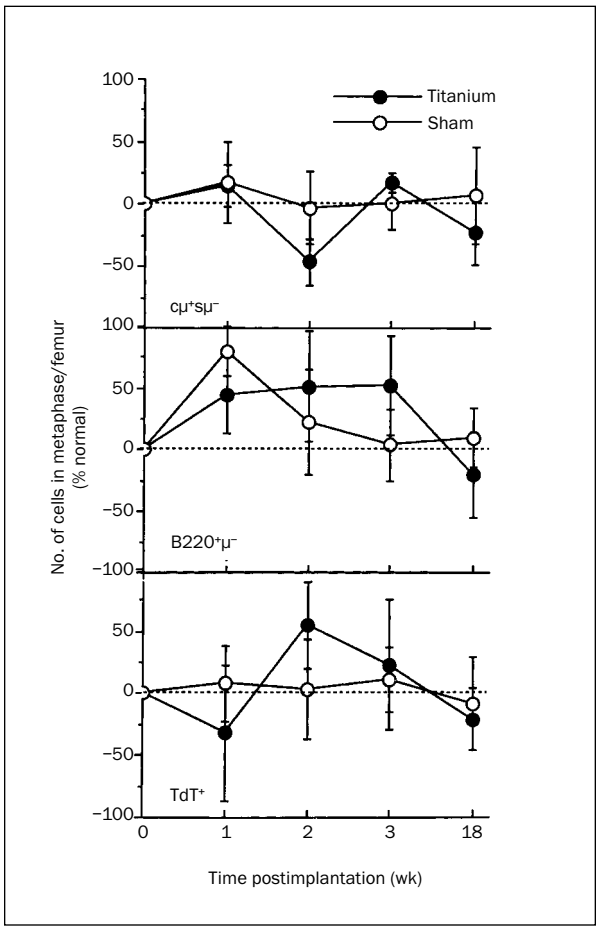

Fig 3 The number of B cell precursors arrested in metaphase after the administration of vincristine sulfate in the bone marrow of mouse femoral shafts following either the surgical placement of titanium implants or a sham operation. Values are expressed as the number of cells in metaphase per femur relative to that found in respective untreated contralateral femora, represented by the zero reference line. The incidences of $c\mu^+$ pre-B cells and $B220^+\mu^-$ and TdT^+ progenitor cells in metaphase were derived from 2,000 to 4,000 nucleated cells and combined with total bone marrow nucleated cellularity to give the number of cells per femur. Each point is based on groups of 3 to 4 mice.

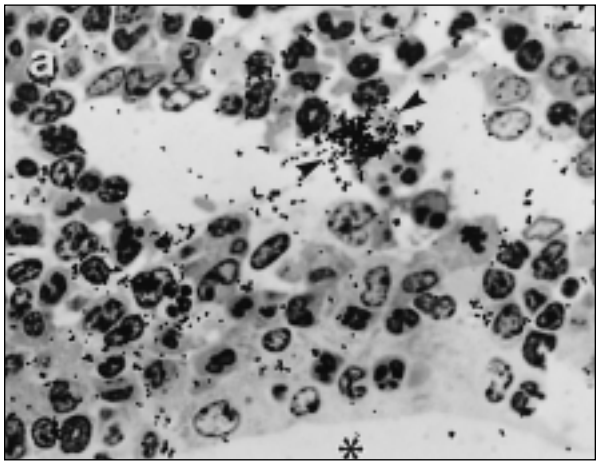

Fig 4a Light microscopic radioautograph of $B220^+$ cells in bone marrow from a femur bearing a titanium implant for 18 weeks, showing labeled cells *(arrowheads)* located in the bone marrow parenchyma near the site previously occupied by the titanium implant *(*)* (magnification ×400).

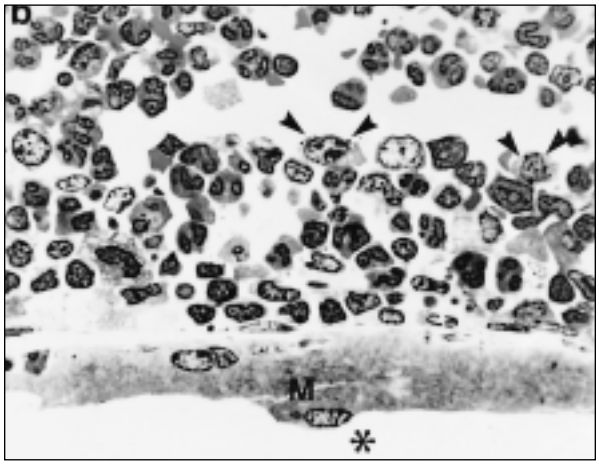

Fig 4b Light microscopic radioautograph of DNA-synthesizing cells in bone marrow from a femur implanted with titanium for 78 weeks, showing labeled cells *(arrowheads)* located in the bone marrow parenchyma near the site previously occupied by the titanium implant *(*)* and a multinucleated giant cell (M) (magnification ×400; alum/iron hematoxylin stain).

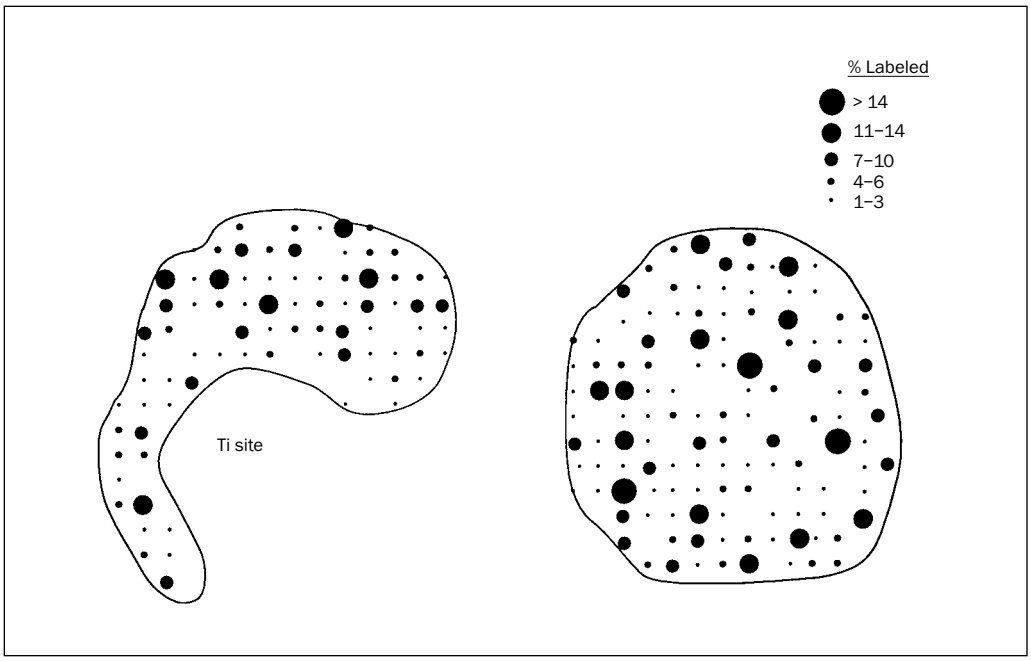

Fig 5a Distribution of labeled B220⁺ cells in cross sections of femoral bone marrow at 18 weeks postimplantation *(left)* and in contralateral limb *(right)*. The circle size reflects the incidence of labeled cells in successive square fields (2,025μ²) across the entire section.

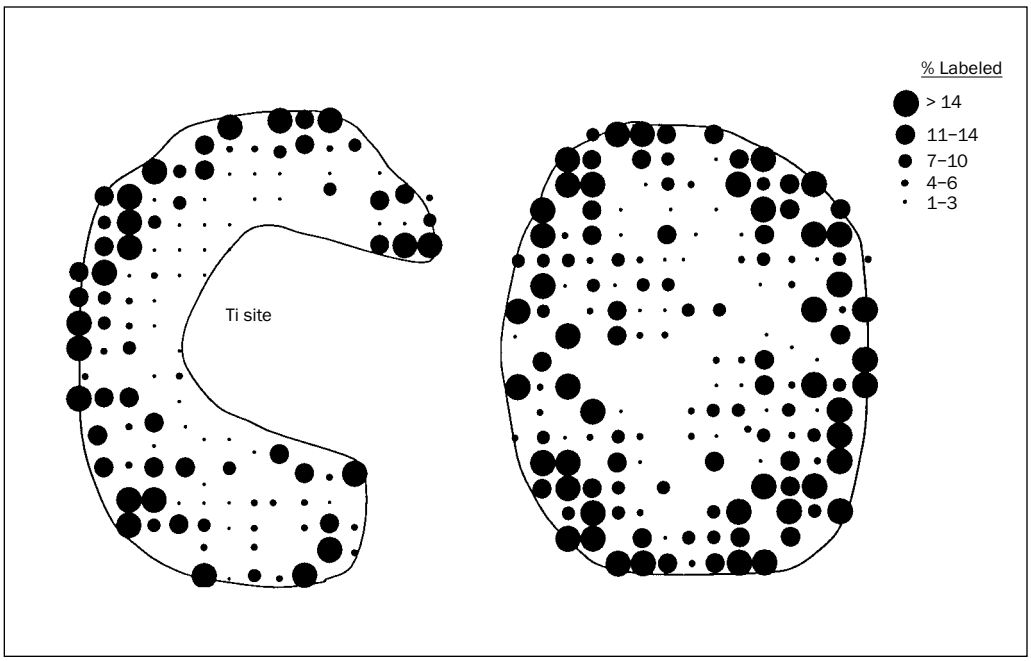

Fig 5b Distribution of labeled DNA-synthesizing cells at 78 weeks postimplantation *(left)* and in contralateral limb *(right)* (Ti site; area occupied by titanium implant). The circle size reflects the incidence of labeled cells in successive square fields (2,025μ²) across the entire section.

DISCUSSION

The present work demonstrates that placement of a titanium implant within mouse bone marrow has no unequivocal long-term effects on either the incidence or the proliferative activity of B lymphocytes and their progenitors in the marrow cavity. The location of both B220+ B lineage cells and dividing cells of all lineages in the bone marrow remains unaltered, despite direct contact between bone marrow components and the implant surface for prolonged periods of time. The evidence so far suggests that titanium implants become effectively myelointegrated, since the normal bone marrow cytoarchitecture seems to be reconstituted both throughout the medullary cavity and in close proximity to the implant surface.

The suitability of using miniaturized femoral implants in the mouse to examine the relationship between titanium implants and bone marrow has been previously demonstrated.[4] Following surgical placement of the implant, 2 phases of myelointegration may be distinguished. At first, local disruption of the delicate bone marrow structure, with its rich network of blood sinusoids, is repaired and repopulated with lymphohemopoietic cells. Secondly, the regenerated bone marrow cells coexist with the intramedullary titanium surface for extended periods of time. The present study confirms that the bone marrow initially regenerates to fill the marrow cavity completely. This brings a morphologically normal mixture of parenchymal cells into contact with the titanium surface, there being no apparent barrier of extracellular matrix or abnormal cells, with the notable exception of periodic multinucleated giant cells applied to the implant surface. The length of time over which the implant-marrow interface persists without the growth of intervening tissues—at least 1.5 years in mice and probably for life—makes it important to verify the long-term biocompatibility of the titanium surface with respect to the local processes of lymphohemopoietic cell proliferation and differentiation.

Stages of B cell differentiation in bone marrow as an index of myelointegration have been examined because of the practical importance of the risk that sustained perturbations of precursor B cells undergoing Ig gene rearrangement may lead to B cell abnormalities. Among the short-term fluctuations noted in precursor B cell populations, the most striking is an increase in the number of cμ+ pre-B cells to 75% above normal values at 1 week postimplantation, followed by subnormal values at 3 weeks. Similar oscillations occur after other perturbations. Sublethal gamma irradiation produces an initial depletion of cμ+ pre-B cells in mouse bone marrow, followed after 1 week by a pronounced overshoot in their numbers, before returning to normal.[6,35] The mechanisms underlying the transient increase in pre-B cells following titanium implantation remain to be determined. Possibly, local repair processes and the systemic effects of surgery may evoke the production of both local and circulating cytokines, including interleukin-1 (IL-1), IL-4, IL-6, IL-7, and transforming growth factor-beta, all of which can modify cell regulation in the bone marrow.[8,9,24]

In mice with established titanium implants (18 weeks), the B cell populations in the medullary cavity of implanted femora are essentially normal. The most important defined phenotype is the population of cells expressing TdT and undergoing Ig heavy chain gene rearrangement. Although their numbers appear to be somewhat increased, their total proliferative activity is normal, suggesting that they are not subject to long-term stimulation.

The events occurring in the medullary cavity as a whole, as assayed in bone marrow cell suspensions, may not necessarily reflect those occurring locally at the implant-marrow interface. Therefore, an in situ labeling technique has been adopted to quantitate the spatial distribution of B lineage cells and cycling cells in the intact tissue across entire sections of bone marrow from mice harboring long-term implants (ie, for 18 to 78 weeks).

In vivo radiolabeling by intravenously administered ^{125}I-antibodies provides a sensitive technique to examine the localization of B lineage cells in mouse bone marrow, as visualized by light and electron microscope radioautography.[6,28,36] The method labels target cells in situ without any prior fixation or loss of antigenicity. In normal femoral cross sections, the overall distribution of B220+ B lineage cells is apparently random, but proliferating early precursor B cells are located mainly near the endosteal surface of cortical bone, while sμ+ B lymphocytes are concentrated toward the center of the marrow.[28,36] Other dividing hemopoietic progenitor cells are also found mostly in the peripheral region of the bone marrow,[27,28] indicating specialized microenvironments. The findings suggest a centripetal movement of cells as they differentiate and finally migrate into the blood stream. The present results in control contralateral bone marrow are consistent with previous findings on the localization of B lineage cells and dividing cells. The presence of long-term titanium implants does not perturb either the normal proliferative gradient across the marrow cavity or the distribution of B220+ B lineage cells. There is no proliferative reaction at the titanium-implant interface. The

interface itself appears not to reconstitute a normal bone-lining cell layer, but rather abuts directly into the central area of the medullary cavity. Many apparently normal hemopoietic cells lie in contact with the titanium surface, with no visible barrier between. In addition, however, certain multinucleated giant cells become located between the implant surface and the lymphohemopoietic compartment.

The findings suggest that the local microenvironmental organization of cells remains unaffected by the presence of either the osseointegrated titanium implant or the multinucleated giant cells at the interface. Giant cells belong to the monocyte-macrophage lineage and generally characterize chronic inflammatory processes. The significance of the persisting macrophages and giant cells at the implant surface remains to be determined.

Although materials used in implants appear to be inert, they can evoke biologic responses in particulate form. Local and systemic reactions secondary to implant debris can occur following mechanical wear in orthopedic appliances, resulting in osteolysis and implant loosening.[37,38] Titanium alloy particles (4.0 μm in diameter) placed into bone marrow of rabbit tibiae are ingested by scattered macrophages.[39] Immunohistochemical labeling of tissues containing particulate titanium surrounding failed hip replacements demonstrates increased numbers of macrophages and T lymphocytes but an absence of B lymphocytes.[40] Thus, the giant cells persisting at the implant-marrow interface in the present work may represent a response to materials shed from the implant interface. In vitro ingestion of titanium alloy particles by macrophages induces the release of inflammatory mediators that are implicated in osteolysis and hemopoietic regulation, including IL-1, IL-6, prostaglandin E_2, and tumor necrosis factor.[41] Exposure of mononuclear cells to metal surfaces in vitro results in elevated levels of IL-1 and tumor necrosis factor.[42]

Chronic macrophage activation in peripheral tissues of the body can cause increased proliferation of early precursor B cells in the bone marrow, mediated by soluble factors, of which IL-1 is a candidate.[22,24–26] In the present work, however, the lack of increased incidence of ^{3}H-TdR labeled cells or long-term changes in precursor B cell population dynamics around giant cells seemingly eliminates the possibility that they are providing a proliferative stimulus to cells in the immediate vicinity. It is not excluded that they may actually suppress local cell proliferation. It remains to be determined whether giant cells at the titanium implant interface secrete cytokines, which may affect some other aspects of cell functioning or cell death[43] in the adjacent bone marrow.

SUMMARY

In general, after recovering from the initial trauma resulting from the placement of a titanium implant through cortical bone into the medullary cavity, the bone marrow in mice maintains a long-term interface with the implant surface. Despite the persistence of multinucleated giant cells at the interface, a harmonious state of myelointegration appears to have been established. The criteria used in the present work provide no evidence to suggest that titanium implants in contact with mouse bone marrow carry a long-term risk of local hemopoietic perturbations or marrow-derived B lineage neoplasms. Further studies at clinical implant sites will be required before complete confidence about myelointegration in humans can be assumed.

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