

Bone Marrow-derived Human Hematopoietic Stem Cells Engraft NOD/SCID Mice and Traffic Appropriately to an Inflammatory Stimulus in the Joint

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ABSTRACT. Objective. Studies of human inflammatory arthritis would be significantly aided by the development of better animal models. Our hypothesis is that it is possible to develop humanized arthritis models through novel techniques of hematopoietic stem and progenitor cell (HSPC) delivery.

Methods. Bone marrow was obtained from patients with osteoarthritis who were undergoing total hip replacement. HSPC were enriched by negative selection and injected into the femur of irradiated anti-CD122 treated nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Human cell engraftment was analyzed by flow cytometry. Arthritis was induced by an intraarticular injection of *Chlamydia trachomatis* and injected knee joints were examined 5 days later by histology and immunohistochemistry.

Results. Human bone marrow HSPC successfully engrafted NOD/SCID mice, with some mice showing up to 90% engrafted human cells. Human B lymphoid and myeloid cells were detected in the bone marrow and spleen 6 weeks following transfer of HSPC, and engrafted recipient mice remained healthy up to 12 weeks postinjection. *Chlamydia*-injected mice that had been repopulated with HSPC had synovial inflammation, consisting of human neutrophils and macrophages.

Conclusion. Bone marrow-derived human HSPC engraft NOD/SCID mice and traffic appropriately to an inflammatory stimulus in the joint, thus offering the potential for direct studies on the immunopathogenesis and treatment of human arthritis. (J Rheumatol First Release Feb 1 2010; doi:10.3899/jrheum.090317)

Key Indexing Terms:

HEMATOPOIETIC STEM CELL

ARTHRITIS

NOD/SCID MICE

A rigorous analysis of the immune basis of inflammatory joint diseases in patients has been limited by ethical and technical constraints. Yet animal models that faithfully recapitulate the cellular events of these diseases have also proved difficult to develop. Hematopoietic stem and pro-

genitor cells (HSPC) are pluripotent cells that are capable of repopulating the immune system and thereby reestablishing the immune repertoire of the donor. Nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice have been shown to accept human HSPC and may allow the engraftment of the full spectrum of immunocompetent cells. Our hypothesis was that immune events leading to development of human arthritis can be recapitulated in NOD/SCID mice by transfer of HSPC. As proof of principle we used HSPC from patients with osteoarthritis (OA) to address the feasibility of using HSPC from adult patients to repopulate the immune system of NOD/SCID recipient mice.

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MATERIALS AND METHODS

Patient samples and CD34+ cell enrichment. Bone marrow was obtained from patients with OA who were undergoing total hip replacement. Cells and bone fragments were diluted in phosphate buffered saline containing sodium citrate and 2% fetal calf serum, shaken for 30 min and filtered through a cell strainer. Red blood cells (RBC) were removed and CD34+ HSPC enriched by negative selection using the StemSep system (StemCell Technologies, Vancouver, BC, Canada).

The study was approved by the Research Ethics Board of the University Health Network (UHN, Toronto, Ontario, Canada). Patients provided consent preoperatively for use of tissue obtained at surgery.

Bone marrow reconstitution of NOD/SCID mice. Mice were bred in the ani-

mal facility at the UHN from breeding pairs originally purchased from Taconic (Hudson, NY, USA). Mice were irradiated with 3.75 Gy and injected intraperitoneally with 200 μ g anti-CD122 antibody 24 h before HSPC transplantation. NOD/SCID mice, aged 8 to 10 weeks, were injected in the right femoral cavity with $1-5 \times 10^5$ CD34-positive-enriched HSPC. The monoclonal anti-CD122 antibody was generated from the hybridoma cell line TM-B1 (a gift from T. Tanaka, Osaka University Medical Center, Osaka, Japan) and purified over a HiTrap Protein G HP column (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Flow cytometry. Bone marrow cells were isolated from mice by flushing the femoral, tibial, and pelvic bones, with the right and left sides being isolated independently. For mice that received an intraarticular injection, the knee joint was removed prior to flushing. Flow cytometry using a dual-laser FACSCalibur instrument (BD Biosciences, San Jose, CA, USA) was used to analyze $1-5 \times 10^5$ RBC-depleted cells from the bone marrow or spleen, stained with various combinations of directly conjugated monoclonal antibodies (mAb). Results were analyzed using CellQuest software. The following mAb were purchased from BD Biosciences Pharmingen: fluorescein isothiocyanate conjugated anti-CD3, anti-CD4, and mouse IgG;

phycoerythrin (PE) conjugated anti-CD8, anti-CD19, anti-CD38, and mouse IgG; and allophycocyanin conjugated anti-CD3, anti-CD4, anti-CD14, anti-CD16, anti-CD45, anti-CD56, and mouse IgG. PE anti-CD33 and PE-Cyanine 5 anti-CD34, anti-CD45, and mouse IgG were purchased from Coulter (Fullerton, CA, USA).

Chlamydia-induced arthritis and immunohistochemistry of the joint. Ten microliters of *Chlamydia trachomatis* (4×10^6 IFU) was injected into both knee joints of mice, with or without prior engraftment with HSPC, 5–6 weeks postirradiation. Mice were sacrificed 5 days later and the knee joints dissected from the rest of the femur and tibia, fixed in formalin, and decalcified. The joints were then sectioned, stained with hematoxylin and eosin, and scored by an observer who was blinded to the experimental manipulations performed, using a histopathological scoring system¹. Five criteria were used to grade the joints: synovitis, inflammatory cell infiltration, joint space exudation, pannus formation, and bone/cartilage damage. Each category was scored 0–4, where 0 = normal and 4 = extreme pathologic change (maximum total possible score = 20). To determine whether human cells had infiltrated the joint, sections were processed to remove paraffin and stained with biotinylated anti-human CD45 antibody, anti-human CD68

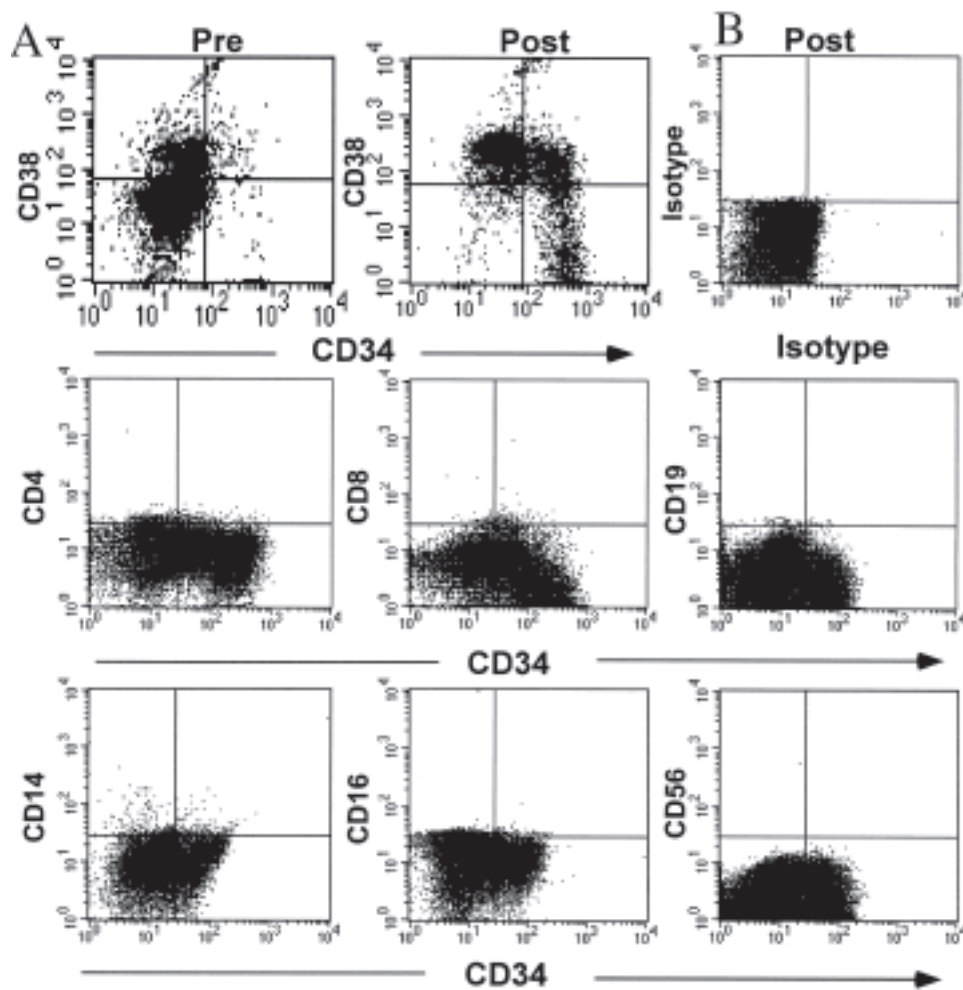
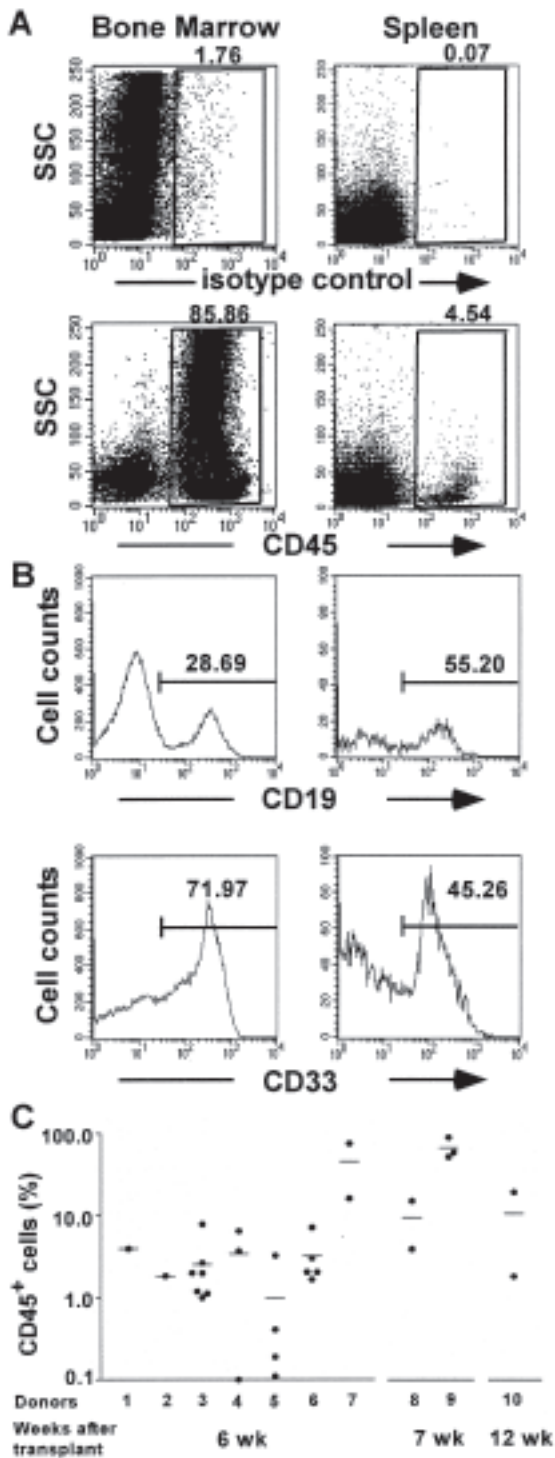


Figure 1. Phenotypic analysis of human Lin-CD34 enriched hematopoietic stem and progenitor cells (HSPC). A. Flow cytometry of bone marrow cells isolated from a patient with OA and stained with anti-CD38 and -CD34 antibodies pre- and post-enrichment with the StemSep system. HSPC cells are CD34+, and CD38 is a marker of HSPC differentiation, with the CD38– population being more primitive. B. Characterization of post-enrichment of Lin-CD34 enriched HSPC. Cells were labeled with monoclonal antibodies against lineage-committed cell markers, such as isotype control, CD4, CD8, CD19, CD14, CD16, and CD56, to reveal the possible contamination cells.



antibody (DakoCytomation, Glostrup, Denmark), anti-human CD20 antibody (Novocastra, Newcastle, UK), or antimouse Ly6G antibody as a control (BD Biosciences). Antibody staining was demonstrated with streptavidin-peroxidase.

Statistical analysis. Data are expressed as the mean \pm standard deviation (SD). Statistical analysis was performed using the Mann-Whitney nonparametric test for comparisons between joint scores.

Figure 2. CD34⁺ HSPC enrichment from osteoarthritis (OA) bone marrow cells and engraftment in NOD/SCID mice. **A.** Engraftment of human cells in the right femoral bone marrow (injected side) and spleen of irradiated repopulated NOD/SCID mice. Cells have been stained with an isotype control or anti-human CD45, a pan-hematopoietic cell marker. Dot plots show regions indicating the positive population and numbers in the top-right corner showing the percentage of positive cells. **B.** Engraftment of human B (CD45+CD19⁺) and myeloid (CD45+CD33⁺) cells in the right femur and spleen 7 weeks after reconstitution. Histograms are shown gated on the CD45⁺ population. Numbers above markers indicate the percentage of positive cells within the CD45⁺ population. **C.** Human cell (CD45⁺) engraftment in the bone marrow of all NOD/SCID mice that received CD34⁺ enriched HSPC from 10 donors with OA at 6, 7, and 12 weeks after transplantation. Each circle represents data from an individual mouse and horizontal bars indicate the mean percentage of CD45⁺ cells in each mouse from 1 donor.

RESULTS

Repopulation of NOD/SCID mice by OA HSPC. HSPC were isolated from 18 patients with OA (ages 26 to 76 yrs; mean 54.72 ± 13.76). On average, 272×10^6 bone marrow cells were obtained, which, following negative selection using the StemSep system, yielded roughly 4×10^6 cells, which were 27.8%–83.7% (mean $45.38 \pm 15.50\%$) CD34-positive (Figure 1A). This system specifically depletes CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, and glycoprotein A-expressing cells. As shown in Figure 1, residual contaminating cells were mostly CD38⁺CD34[−] and did not stain with cell-surface markers for B cells (CD19⁺), T cells (CD4⁺ or CD8⁺), natural killer cells (CD56⁺), or macrophages (CD14^{high}CD16⁺). However, there was a small proportion of residual CD14⁺ monocytes, which represented ~0.01% of the purified population (resulting in 10–50 transferred cells per mouse). Studies have demonstrated that the CD38⁺CD34[−] population consists of primitive cells that are incapable of repopulating the myeloid and lymphoid lineages in NOD/SCID mice². Following intrafemoral injection, successful engraftment of human cells in NOD/SCID recipient mice was demonstrated by positive staining of bone marrow cells from the right femur or splenocytes with an anti-human CD45⁺ antibody (Figure 2A). Human cells of both myeloid (CD45⁺CD33⁺) and B lymphoid (CD45⁺CD19⁺) lineages were seen in recipient mice at 7 weeks post-reconstitution (Figure 2B), but mature CD4 (CD4⁺CD3⁺) or CD8 (CD8⁺CD3⁺) T cells were not detected. HSPC from 10 patients with OA were transplanted into NOD/SCID mice. Figure 2C shows the various levels of human CD45⁺ cell engraftment obtained in each mouse at 6 to 12 weeks post-transplantation. Analysis of both the non-injected, contralateral femoral bone marrow and the spleen also showed engrafted human cells, indicating that OA stem cells migrated through the bloodstream from the injected femur to repopulate diverse sites (data not shown).

HSPC-repopulated NOD/SCID mice develop Chlamydia-induced arthritis. To determine whether human cells can migrate to the joint in response to an intraarticular inflam-

matory stimulus, mice were injected with *Chlamydia trachomatis* in both knee joints. We have shown that at 5 days following injection the predominant inflammatory cells in the joint are macrophages and neutrophils^{3,4}. As shown in Table 1 and Figure 3A, unmanipulated NOD/SCID mice generated an inflammatory response to *Chlamydia* injection, which was abrogated in mice treated with irradiation and anti-CD122 injection. Repopulation of irradiated, anti-CD122-treated mice by human HSPC restored the inflammatory response to *Chlamydia*, indicating that inflammatory cells migrate to the infected joint in the repopulated mice. Migration of inflammatory cells required injection with *Chlamydia*, because only scattered inflammatory cells were seen in the occasional joint of repopulated mice that had not been injected with *Chlamydia*.

Human cells migrate to the inflamed synovium. Mice that were irradiated but not transplanted with HSPC had no inflammatory cells in their joints and demonstrated no staining with anti-human CD45 (data not shown). Although inflammation was seen in infected nonirradiated joints, only anti-mouse Ly6G+ cells were present in the bone marrow and joints (Figure 3B and 3C). In irradiated and repopulated mice, very few antimouse Ly6G+ cells were seen and there was positive staining with anti-CD45 mAb for human cells in both the joints and bone marrow (Figure 3D and 3E). Most of the CD45+ cells within the joint also stained with anti-human CD68 antibody, indicating the presence of human macrophages in the joint with only occasional CD20+ B cells in some joints (Figure 4). None of the CD45+ cells had the morphologic characteristics of granulocytes. Thus human cells can migrate to the inflamed synovium following a local *Chlamydia* challenge.

DISCUSSION

This is the first demonstration that HSPC from adult human bone marrow can successfully engraft NOD/SCID mice and that these expanded human immune cells traffic appropriately to sites of joint inflammation. Although engraftment of NOD/SCID mice with human adult bone marrow-derived HSPC has been demonstrated, these studies have most commonly used cord blood HSPC because of difficulties with

reconstitution⁵⁻⁷. However, recent technologic improvements, such as blockade of the interleukin 2 (IL-2) receptor by the administration of anti-CD122 antibody, and with intrafemoral injection, have improved engraftment^{5,8}. Here, we show that these approaches can be used to successfully reconstitute mice with HSPC derived from the bone marrow of adult patients, even in their seventh decade, resulting in multilineage myeloid and lymphoid cells, and raising, for the first time, the possibility of using HSPC derived from patients with arthritis to generate humanized mouse models to study these conditions.

Our results further demonstrate that the expanded cells derived from adult HSPC are functional and migrate appropriately to inflammatory signals in the joint. While this is an important proof of principle for the generation of humanized mouse models of arthritis, there remain some hurdles to overcome before this technique can be applied to the study of rheumatoid arthritis and ankylosing spondylitis, 2 conditions for which informative animal models are urgently required. Although stem cells from adult bone marrow engrafted and expanded in NOD/SCID mice with a similar repopulation profile to that of cord blood-derived stem cells⁵⁻⁷, mature T cells were absent. As the conditions for autoimmune disease are generally considered to be at least in part T cell-derived, further efforts will be needed to achieve T cell reconstitution. Several groups have demonstrated that T cells can develop in NOD/SCID IL-2 receptor common γ -chain knockout mice transplanted intrahepatically as newborns⁹⁻¹². T cells in these mice show appropriate MHC-restricted responses and can provide support for antibody production¹³⁻¹⁵. These mice were not generally available at the time of our study, but could be used in future studies. Alternatively, *in vitro* culture techniques, such as co-culture with Delta-like-1, which have been shown to improve T cell development, could be used prior to bone marrow transfer to augment T cell engraftment¹⁶. Nevertheless, our experiments indicate that a humanized mouse model can be generated to assess the early inflammatory response in the joint and further suggest that if T cell engraftment can be achieved, such cells may similarly traffic appropriately to joints^{17,18}.

Table 1. Joint scores following *Chlamydia* injection into NOD/SCID mouse knee joints with various experimental conditions.

Condition	Irradiation	Anti-CD122 Antibody	HSPC	<i>Chlamydia</i>	No. Mice	No. Donors	Mean Scores
1	–	+	–	–	5	0	0.58 ± 1.43
2	–	+	–	+	3	0	6.17 ± 1.04
3	+	+	–	+	5	0	0.00 ± 0.00
4	+	+	+	–	7	4	0.36 ± 0.48
5	+	+	+	+	16	7	2.84 ± 3.48*

Similar scores were obtained for the right and left joint so that mean joint score is shown. * p = 0.028 vs condition 4 and p = 0.0164 vs condition 3. NOD/SCID: nonobese diabetic/severe combined immunodeficiency; HSPC: hematopoietic stem and progenitor cell.

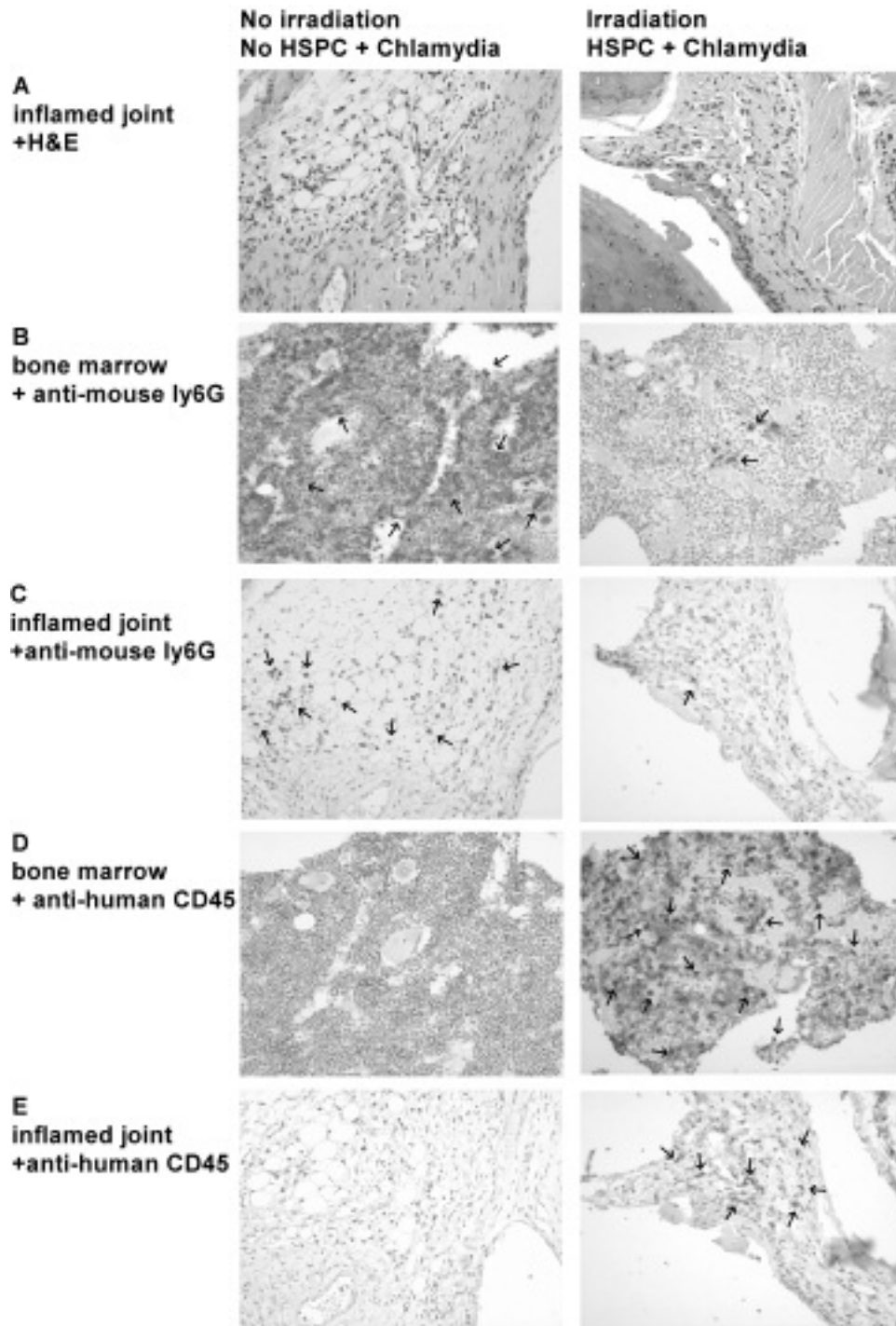


Figure 3. CD45+ human cells migrate to inflamed mouse joints. Joint histopathologic changes on day 5 after *Chlamydia* injection for nonirradiated, nontransplanted mice (left panels) or irradiated, hematopoietic stem and progenitor cell (HSPC) repopulated NOD/SCID mice (right panels). Both bone marrow and inflamed joints of these 2 representative mice are shown. A. Mouse joints stained with H&E showing inflamed synovial joint tissue. Synovial hyperplasia and hypertrophy with some mononuclear cell infiltration are seen in the joints of both mice. Original magnification $\times 20$. B and C. Mouse bone marrow and joints stained with anti-mouse Ly6G (mouse granulocyte-specific) antibody. Original magnification $\times 20$. D and E. Mouse bone marrow and joints stained with anti-human CD45 (pan-hematopoietic cell marker) antibody to detect the presence of repopulated human cells in the bone marrow and inflamed joint. Original magnification $\times 20$.

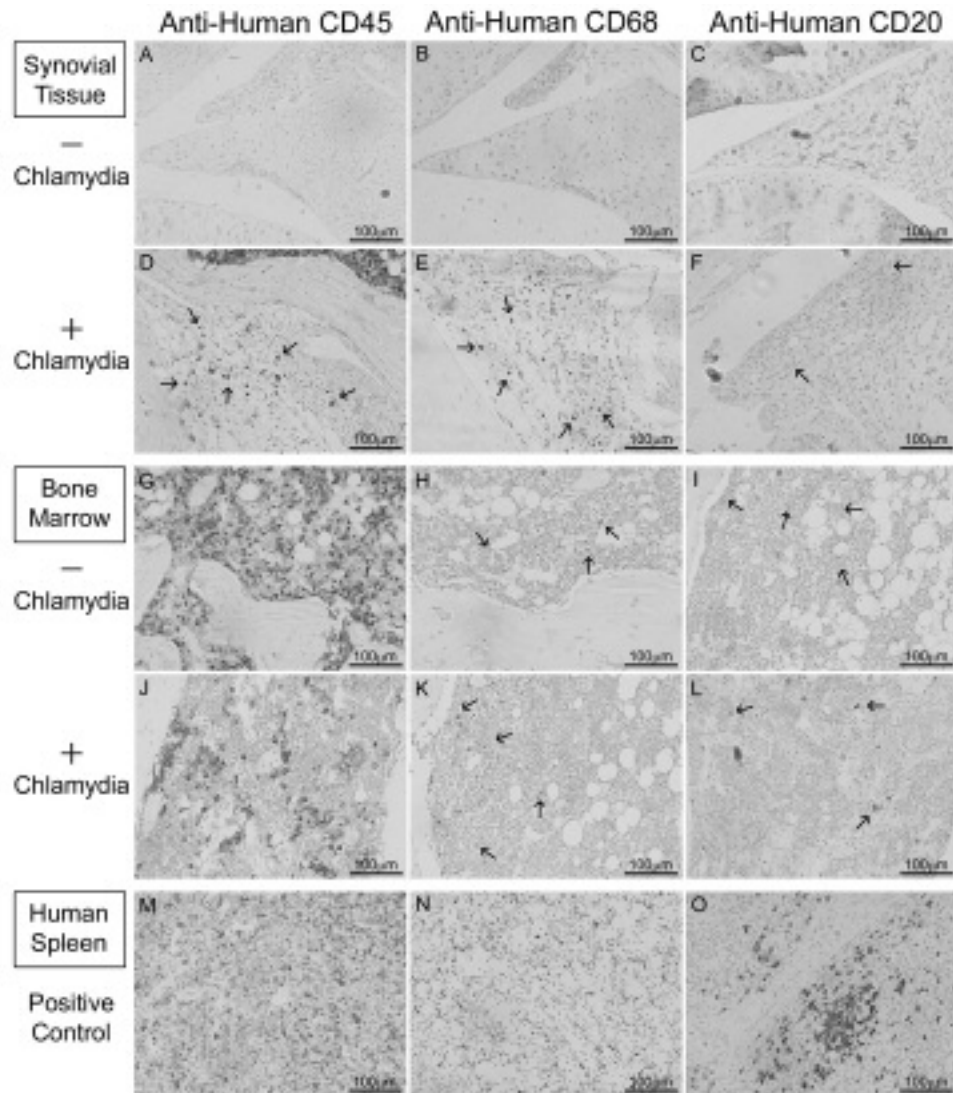


Figure 4. Inflamed mouse joints infiltrated with human CD45+, CD68+, and CD20+ cells with *Chlamydia* injection. Compare mouse joints (A–F) and bone marrows (G–L) with or without *Chlamydia* injection stained with anti-human CD45+, CD68+, and CD20+ antibodies. Without *Chlamydia* injection, mouse joints show normal joint structure and no infiltration of human CD45+, CD68+, and CD20+ cells (A–C), although engrafted human cells, CD45+, CD68+, and CD20+ cells can be found in the bone marrow (G–I). With *Chlamydia* injection, mouse joints show inflamed synovial tissue and human CD45+, CD68+, and CD20+ cells are found in the joints (D–F) and engrafted in the bone marrow (J–L). Antibodies against human CD45+, CD68+, and CD20+ shown as positive controls were stained with human spleen tissue (M–O).

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