IL-7 induces bone loss *in vivo* by induction of receptor activator of nuclear factor κB ligand and tumor necrosis factor α from T cells

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IL-7, a powerful lymphopoietic cytokine, is elevated in rheumatoid arthritis (RA) and known to induce bone loss when administered *in vivo*. IL-7 has been suggested to induce bone loss, in part, by stimulating the proliferation of B220$^+$ cells, a population capable of acting as early osteoclast (OC) precursors. However, the mechanism by which IL-7 leads to differentiation of precursors into mature OCs remains unknown. We previously reported that, *in vitro*, IL-7 up-regulated T cell cytokines including receptor activator of nuclear factor κB ligand (RANKL). To demonstrate the importance of T cells to the bone-wasting effect of IL-7 *in vivo*, we have now examined IL-7-induced bone loss in T cell-deficient nude mice. We show that T cell-replete mice undergo significant osteoclastic bone loss after IL-7 administration, concurrent with induction of RANKL and tumor necrosis factor α (TNF-α) secretion by splenic T cells. In contrast, nude mice were resistant to IL-7-induced bone loss and showed no detectable increase in either RANKL or TNF-α, despite an up-regulation of B220$^+$ cells. Importantly, T cell adoptive transfer into nude mice restored IL-7-induced bone loss, and RANKL and TNF-α secretion, demonstrating that T cells are essential mediators of IL-7-induced bone loss *in vivo*.

osteoclast | cytokines | osteoporosis | rheumatoid arthritis

The inflammatory cytokine IL-7 is a potent osteoclastogenic factor that induces bone loss *in vivo* (1). Enhanced levels of IL-7 have long been associated with rheumatoid arthritis (RA) (2–4), a condition known to cause periarticular and systemic bone loss (5).

IL-7-induced bone destruction has been suggested to be mediated by B220$^+$ cells, early B cell precursors, because increased B lymphopoiesis similar to that observed during estrogen (E2) deficiency is induced by IL-7 *in vivo* (1). How IL-7 leads to bone loss via up-regulation of B220$^+$ cells is poorly understood. Although B220$^+$ cells have the capacity to differentiate into osteoclasts (OCs), under the influence of osteoclastogenic cytokines (6), thus increasing the OC precursor pool, the source of the osteoclastogenic cytokines necessary to induce the differentiation of OC precursors (B220$^+$ or monocyte derived) into mature OCs remains to be determined.

We have recently reported that IL-7 induces OC formation *in vitro* by production of receptor activator of nuclear factor κB ligand (RANKL) from T cells (7). Consistent with these data, RANKL derived from activated T cells has been shown to be critical to the bone loss occurring in animal models of RA (8).

Because IL-7 stimulates both B and T lymphopoiesis and both lymphocyte lineages have been implicated in osteoclastogenesis (6, 8–13), the issues of how IL-7 causes bone loss, and the cell populations involved, remain to be delineated.

The objective of this study was to demonstrate conclusively that, *in vivo*, T cells are critical mediators of the bone wasting effect of IL-7 by using a genetic model of T cell deficiency, the nude mouse.

Materials and Methods

Animal procedures were approved by the Washington University Animal Studies Committee. All reagents were from Sigma unless otherwise specified.

T Cell Adoptive Transfer and IL-7 Administration. Purified T cells were injected i.p. into athymic (−/−) nude recipients on the C57BL/6 background (Taconic Farms) as described (14). Mice (seven mice per group) were injected daily for 30 days with vehicle (PBS) or 10 μg/kg body weight rhIL-7 (Sanofi, Paris).

Flow Cytometry. Flow cytometry was performed on a three-color Becton Dickinson flow cytometer (FACScan), by using FITC-conjugated CD3 antibody for T cells, CD19 antibody for B cells, and B220 antibody for early B cell precursors (PharMingen) as described (14). Nonspecific staining was assessed by using isotype-matched controls.

Peripheral Quantitative Computed Tomography (pQCT). Trabecular bone mineral density (BMD) was determined by pQCT using a XCT-960M scanner (Norland Medical System, Fort Atkinson, WI) as described (9, 14). Data points represent the average of seven mice per group (average of four trabecular slices per mouse) ± SEM. A separate group of mice were killed before the start of the experiment to establish baseline BMD.

Dual-Energy X-Ray Absorptiometry (DEXA). BMD was determined in intact mice at baseline and at 4 weeks by using a PIXImus2 scanner (Lunar, Madison, WI). Anesthetized mice were placed on the imaging tray in a prostrate position. Data points represent the average of both femurs for each group of mice ± SEM and are expressed as percent change from baseline to control for changes in bone formation. Short-term *in vitro* reproducibility was determined to be 1.7%.

Collagen C-Terminal Telopeptide ELISA. Collagen C-terminal telopeptide secretion was measured in the serum of all groups of mice at sacrifice by commercial ELISA (RatLaps, Osteometer Biotechnology, Herlev, Denmark).

RANKL, Tumor Necrosis Factor α (TNF-α), and Osteoprotegerin (OPG) ELISAs. Soluble murine RANKL, TNF-α, and OPG were measured by commercial ELISAs (R & D Systems) in 48-h condi-

Abbreviations: BMD, bone mineral density; CM, conditioned medium; DEXA, dual-energy X-ray absorptiometry; E2, estrogen; OC, osteoclast; OPG, osteoprotegerin; pQCT, peripheral quantitative computed tomography; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor κB ligand; TNF-α, tumor necrosis factor α; TRAP, tartrate-resistant acid phosphatase.

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CELL BIOLOGY

tioned medium (CM) generated from 5×10^6 total white spleen cells per ml from all groups of mice. Triplicate cultures of spleen cells derived from seven mice per group were analyzed for the presence of soluble RANKL or TNF-α. No RANKL, TNF-α, or OPG was detected in culture medium alone.

OC Formation Assay. OCs were cultured from immunomagnetically purified CD11b or $B220^+$ IgM^- precursors (1×10^5 cells per well) stimulated with spleen CM from all groups of mice (see ELISA above). TNF-α-neutralizing antibody (100-fold excess) and/or the RANKL inhibitor OPG (1,000-fold excess), both from R & D Systems, was added to additional wells treated with CM derived from euthymic (+/−) mice treated with IL-7 or athymic (−/−) mice reconstituted with T cells and treated with IL-7. After 10–15 days of culture, cells were stained for tartrate-resistant acid phosphatase (TRAP) and the number of mature OC (TRAP positive and ≥3 nuclei) was counted. In recent *ex vivo* studies of nude mice, we have verified that >98% of TRAP-positive cells with more than three nuclei express pp60c-src, cathepsin K, and calcitonin receptors, and form resorption pits *in vitro*, verifying these cells as functional osteoclasts (9, 14). A similar characterization has been reported for $B220^+$ cell-derived OCs (6).

Statistical Analysis. Cross-sectional BMD and OC numbers *in vivo* and OC formation by CM *in vitro* were analyzed by ANOVA and Fisher's protected least significant difference tests. $P \leq 0.05$ was considered significant. IL-7-induced RANKL and TNF-α production was analyzed vs. not detected by using Fisher's exact test. Group mean values were compared by two-tailed Student's *t* test and Fisher's exact test.

Results

IL-7 Does Not Up-Regulate Bone Resorption and Bone Loss in T Cell-Deficient Nude Mice *in Vivo*. To investigate whether T cells are essential to the mechanism by which IL-7 induces bone loss *in vivo*, we injected IL-7 into euthymic (+/−) control and genetically matched athymic (−/−) T cell-deficient nude mice. After daily injection of IL-7 for 4 weeks, the trabecular bone density of the tibias was measured by using pQCT, a specific and sensitive technique for analysis of the trabecular bone compartment. The data show (Fig. 1*a*) that euthymic (+/−) mice injected with IL-7 display a lower BMD (−15%) compared with vehicle-treated mice. In support of a role of T cells, genetically matched T cell-deficient (−/−) nude mice did not undergo bone loss after IL-7 injection. Furthermore, nude mice reconstituted with T cells by means of adoptive transfer (15) again undergo bone loss after IL-7 administration. To avoid the spontaneous T cell regeneration known to take place in mature nude mice, our experiments were conducted in young mice 8 weeks of age. In keeping with the high rate of skeletal growth expected in mice of this age, pQCT measurements detected a 10% increase from baseline BMD (157.9 ± 7.6 mg/cm^3; average ± SEM) in vehicle-treated euthymic (+/−) mice over the 30-day duration of the experiment. In contrast, (−/−) nude mice treated with vehicle showed only a 3% increase in BMD from baseline (153.2 ± 4.0 mg/cm^3) over the same period, suggesting a slower rate of growth or an accelerated baseline rate of bone resorption. Although different BMDs between euthymic control and athymic nude mice were not statistically significant at baseline (8 weeks of age), these differences did reach significance by 12 weeks of age ($P < 0.05$).

The pQCT results were verified by DEXA (Fig. 1*b*) using a PIXImus bone densitometer to analyze the femurs of all groups of mice. This technique provides an integrated measurement of cortical and trabecular bone. A 6.5% decrease in BMD was observed in IL-7-injected euthymic (+/−) control mice compared with vehicle-treated mice. Athymic (−/−) nude mice

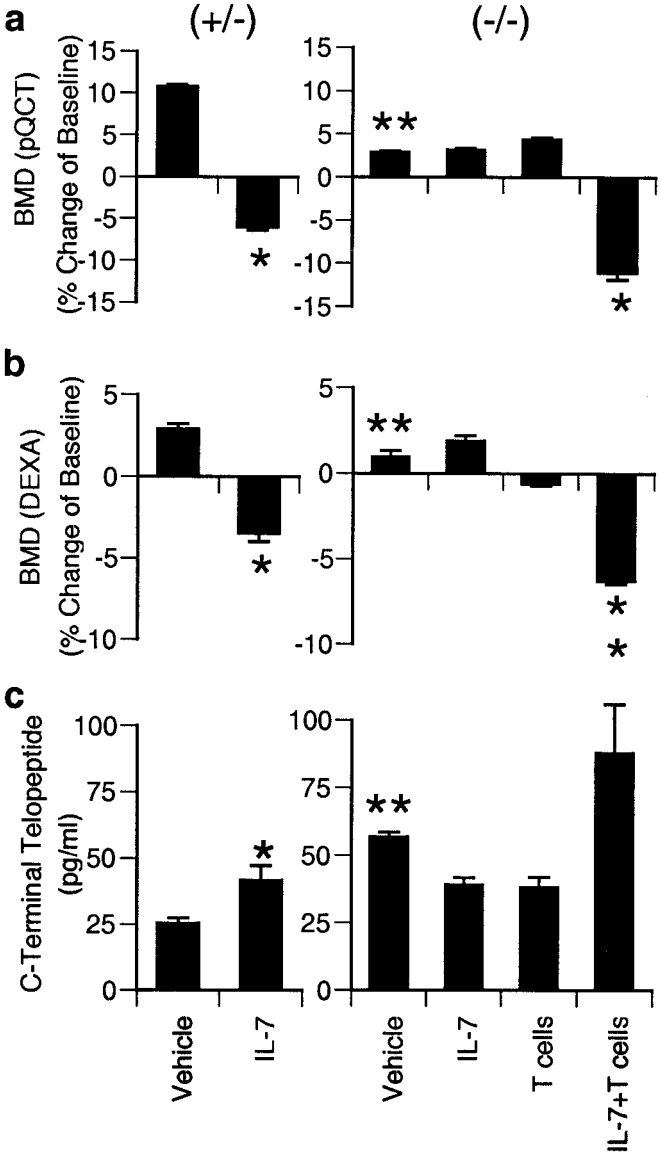

Fig. 1. Injection of IL-7 does not up-regulate bone resorption and bone loss in T cell-deficient nude mice. Euthymic (+/−) control and athymic (−/−) T cell-deficient nude mice or T cell-reconstituted nude mice were injected IP with vehicle (PBS) or IL-7 (10 μg/kg body weight) for 30 days, and BMD was determined by pQCT (*a*) or DEXA (*b*). pQCT was conducted on isolated tibias at sacrifice. Data points represent an average of four trabecular slices per mouse ± SEM. Data are expressed as percent change in BMD from baseline. DEXA was performed on intact mice at baseline and at sacrifice. Data are expressed as percentage change in BMD from baseline ± SEM. (*c*) Collagen C-terminal telopeptide, a specific biochemical marker of bone resorption, was measured in the serum of all groups of mice at 4 weeks. Data points represent average pg/ml of C-terminal telopeptide ± SEM of triplicate measurements. For *a*–*c*, *, $P \leq 0.05$ with respect to relevant vehicle control; **, $P < 0.05$ with respect to euthymic (+/−) vehicle (ANOVA), $n = 7$ mice per group.

again showed no significant loss of bone in response to either IL-7 or T cell reconstitution alone. However, when mice reconstituted with T cells were stimulated with IL-7 a 7% decrease in BMD compared with vehicle injected mice was detected. Once again a significant difference ($P < 0.05$, *t* test) between baseline BMD (0.0478 ± 0.0005 g/cm^2; average ± SEM) of euthymic (+/−) mice and baseline BMD (0.0315 ± 0.0003 g/cm^2) of athymic nude (−/−) mice was detected.

To verify that IL-7 induces bone loss by a mechanism involving

enhanced bone resorption we measured collagen C-terminal telopeptide concentrations, a specific biochemical marker of bone resorption *in vivo*, in the serum of all groups of mice at sacrifice. The data show (Fig. 1*c*) that injection of IL-7 into euthymic (+/−) control mice resulted in a 60% increase in serum collagen C-terminal telopeptide, indicative of enhanced bone resorption. In contrast, athymic (−/−) nude mice injected with either T cells or IL-7 alone do not to show an increase in collagen C-terminal telopeptide secretion. However, IL-7 again elicited a 65% increase in collagen C-terminal telopeptide after reconstitution of athymic (−/−) nude mice with T cells. Baseline levels of bone resorption were significantly ($P < 0.05$) higher in athymic nude mice than in heterozygous controls, suggesting that the decreased BMD observed in nude mice may be the result of higher basal resorption rates.

Successful T cell reconstitution was confirmed by flow cytometric analysis. Whereas spleens of euthymic (+/−) control mice contained ≈23% T cell, nude mice showed <2% $CD3^+$ cells. After T cell adoptive transfer in athymic (−/−) mice, splenic T cell numbers were elevated to 8% of total white cells (data not shown), a number typical of adoptive transfer (16, 17) and one previously found to be sufficient to support bone loss during E2 deficiency, a T cell-dependent process (14).

To verify *in vivo* OC expansion in IL-7-stimulated T cell-replete mice, histological sections were prepared from tibias from all groups of mice and OCs stained by TRAP (Fig. 2*a*). Quantitation of OCs (Fig. 2*b*) confirms a statistically significant ($P < 0.05$) increase in euthymic (+/−) mice injected with IL-7. In contrast, athymic (−/−) nude mice do not show increased numbers of OCs after IL-7 administration, but again respond to IL-7 after T cell reconstitution. Interestingly, OC formation in vehicle-treated nude mice showed a statistically significant ($P < 0.05$) increase in baseline OC number compared with heterozygous controls.

IL-7-Induced Expansion of $B220^+$ Cells Alone Is Insufficient to Induce Bone Loss. To determine the percentage of $B220^+$ cells in the bone marrow of nude mice under baseline conditions and in response to IL-7, the bone marrow of all groups of mice was analyzed at sacrifice for $B220^+$ B cell precursors and $CD19^+$ mature B cells. The data show (Fig. 3) that nude (−/−) mice contain normal populations of $B220^+$ cells and mature B cells compared with euthymic (+/−) control mice. Consistent with published literature, two populations of $B220^+$ cells (designated M1 and M2) were evident. The $B220^{+low}$ (M1) population represents pre-B cells, whereas the $B220^{+high}$ (M2) population consists of mature B cells (1). Because IL-7 has been reported to selectively elicit an increase in $B220^{+low}$ cells in the bone marrow of normal mice, we verified the effect of IL-7 on $B220^+$ cells in euthymic (+/−) and athymic (−/−) nude mice and found an ≈2-fold increase in $B220^{+low}$ cells in athymic (−/−) nude mice in all groups of mice injected with IL-7 (data not shown). This finding confirms that nude mice undergo a normal expansion of pre-B cells in response to IL-7 and demonstrates that IL-7 induction of B cell precursors alone is insufficient to elicit bone loss in the absence of T cells.

IL-7 Induces RANKL and TNF-α from T Cells *in Vivo*. We have previously reported that, *in vitro*, the major mechanism by which IL-7 induces OC formation is by inducing T cell expression and secretion of the key osteoclastogenic cytokine RANKL (7). In addition, we have recently found that T cell-derived TNF-α is an essential augmenter of RANKL activity in the bone loss associated with E2 deficiency (9, 14). We thus analyzed the concentrations of RANKL, the RANKL inhibitor OPG, a physiological regulator of RANKL activity (18), and TNF-α in CM from equivalent numbers of isolated total white spleen cells, from euthymic (+/−) mice receiving either vehicle or IL-7 or from athymic (−/−) nude mice receiving either vehicle or IL-7 in the presence or absence of reconstituted T cells. Spleen cells were selected rather than bone marrow, because of the comparatively large number of T cells resident in the spleen and the absence of

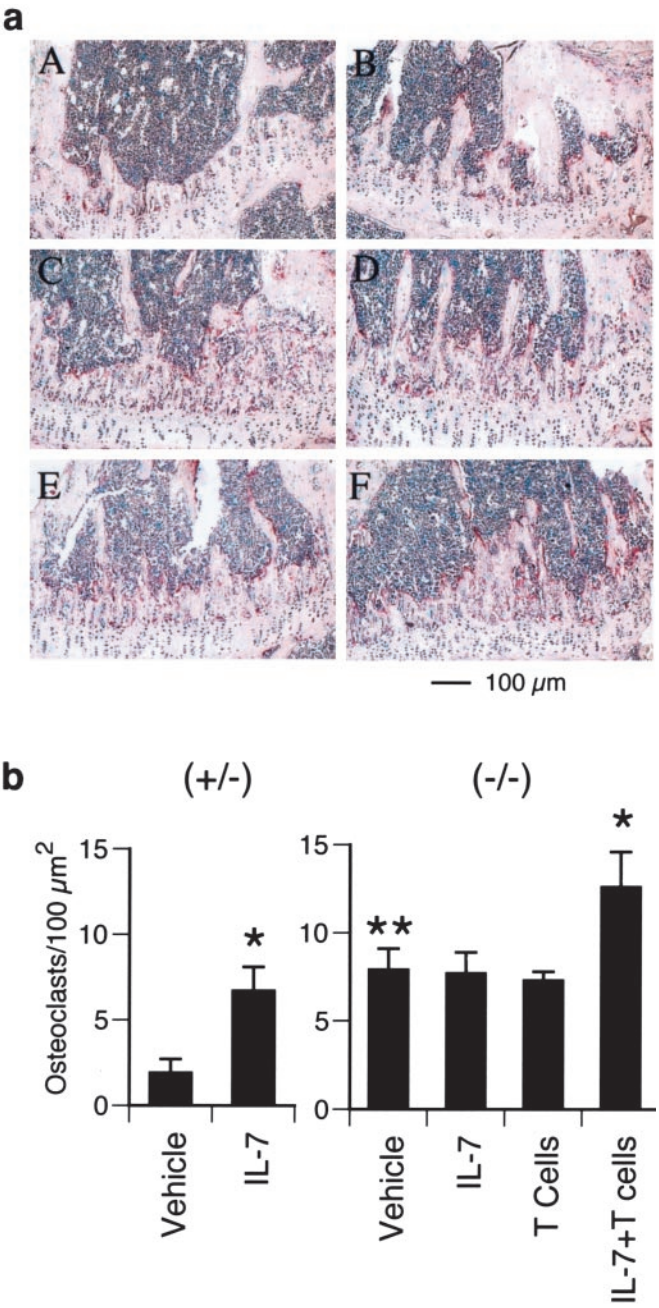

Fig. 2. IL-7 up-regulates OC numbers in T cell-replete but not T cell-deficient mice tibias *in vivo*. (*a*) Euthymic (+/−) control mice were injected IP with vehicle (*A*) or IL-7 (*B*), and athymic (−/−) nude mice were injected with vehicle (*C*), reconstituted with T cells (*D*), injected with IL-7 (*E*), or reconstituted with T cells and injected with IL-7 (*F*) for 30 days, and tibias were fixed in 70% ethanol, paraffin embedded, and sectioned for histology. TRAP-stained OCs (red) from representative sections are shown at ×100 magnification. (*b*) To quantitate OC numbers, TRAP-positive cells were counted by an operator blinded as to the nature of the samples. Ten fields of 100 μm^2, located within the trabecular compartments immediately above the growth plates, were counted for each slide, and the data are presented as the average OC number per 100 $\mu m^2 \pm$ SEM. *, $P < 0.05$ with respect to vehicle-treated mice (ANOVA); **, $P < 0.05$ with respect to euthymic (+/−) vehicle-treated mice (ANOVA).

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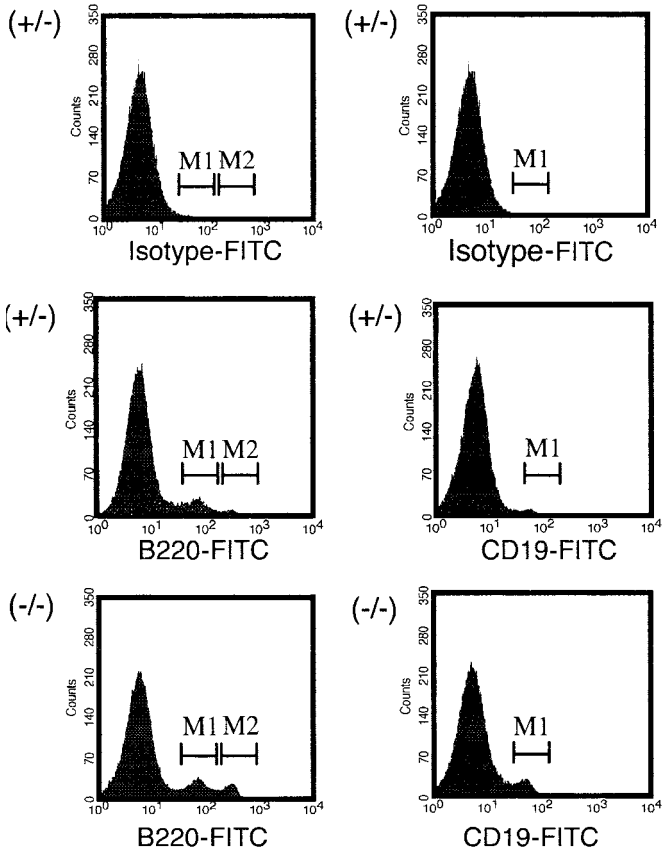

Fig. 3. Athymic (−/−) nude mice contain both B220$^+$ B cell precursors and mature (CD19$^+$) B cells. Bone marrow from untreated euthymic (+/−) and athymic (−/−) nude mice was analyzed for B220$^+$ and mature B cell (CD19) populations by flow cytometry. Two B220$^+$ populations were detected representing B220^{+low} B cell precursors (M1), and B220^{+high} cell populations (M2) representing mature B cells. Cell populations positive for B220^{low} (M1), B220^{high} (M2), and CD19 (M1) were assessed based on comparison to isotype controls. *x* axis units are $\log_{10}$ fluorescence.

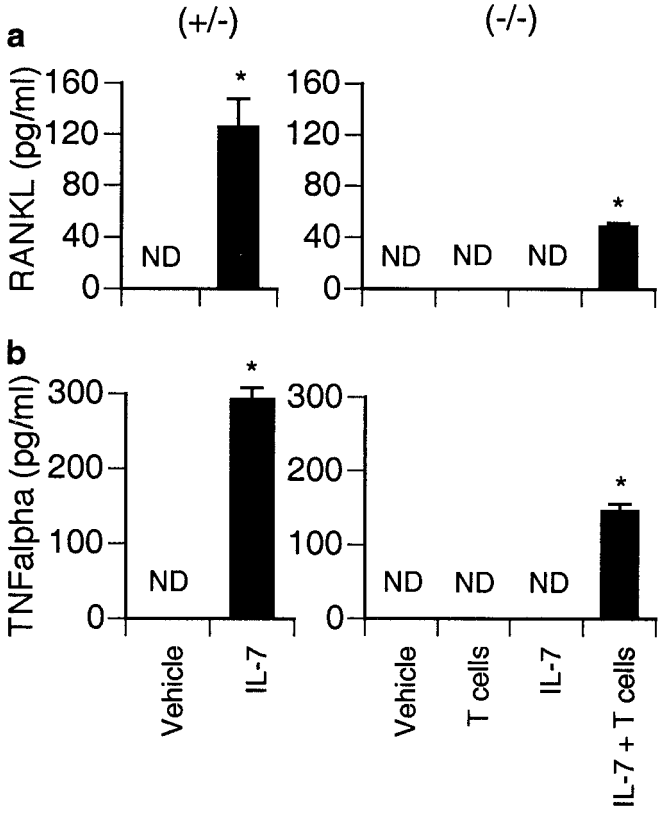

Fig. 4. IL-7 induces RANKL and TNF-α production by IL-7-stimulated T cells *in vivo*. RANKL and TNF-α were determined by specific murine ELISAs in the CM of total white spleen cells from euthymic (+/−) control mice treated *in vivo* with vehicle or IL-7, athymic (−/−) nude mice treated *in vivo* with vehicle or IL-7, and T cell-reconstituted athymic (−/−) nude mice, treated with vehicle or IL-7. Data represent the average of triplicate cultures measured in duplicate ± SEM. *, $P < 0.05$; ND, not detected (Fisher's exact test).

RANKL- and TNF-α-producing stromal cells and osteoblasts, a confounding factor in bone marrow. The data show that IL-7-injected euthymic (+/−) control mice show a large induction of both soluble RANKL (Fig. 4*a*) and TNF-α (Fig. 4*b*) relative to mice receiving vehicle in which no RANKL or TNF-α was detected. In contrast, consistent with the lack of T cells, the major source of RANKL and TNF-α in the spleen, IL-7 failed to elevate either RANKL or TNF-α concentrations in athymic (−/−) nude mice treated with vehicle or IL-7, nor in mice reconstituted with T cells. IL-7 again induced detectable levels of RANKL and TNF-α after T cell reconstitution. OPG was below the level of detection in all groups tested (data not shown).

CMs from IL-7-Stimulated Splenic T Cells Induce OC Formation from Early Precursors of the Monocyte and B220 Lineage. We have previously reported that in an *in vitro* human model of osteoclastogenesis IL-7 induced OC formation by a mechanism that primarily involved the up-regulation of the key osteoclastogenic cytokine RANKL (7). We (9, 14) and others (19–21) have also reported that TNF-α is a powerful augmenter of RANKL activity. Because our ELISA studies above detected both RANKL and TNF-α in splenocyte CM derived from *in vivo* IL-7-stimulated euthymic (+/−) control and athymic (−/−) T cell-reconstituted nude mice, we investigated whether RANKL and/or TNF-α contained in the CM from the different groups of mice were capable of generating OCs when added to early OC precursors. As a source of early OCs precursors we used highly purified CD11b monocytes, a well established early OC precursor (22). In addition, we tested purified B220$^+$ IgM$^-$ cells, early B cell precursors previously reported to be implicated in the bone loss associated with E2 deficiency (1, 23) and recently established to be capable of differentiating along an OC lineage (6).

OCs were generated by treatment with spleen cell-derived CMs from all groups of mice. The data show that T cell CM from IL-7-treated euthymic (+/−) control mice elicited a 2-fold increase in OC formation compared with vehicle-treated mice in both CD11b (Fig. 5*a*) and B220$^+$ (Fig. 5*c*) cells. In contrast, athymic (−/−) nude mice either reconstituted with T cells or injected with IL-7 failed to induce increased numbers of OCs as compared with vehicle-treated mice, irrespective of whether the OC precursors used were CD11b (Fig. 5*b*) or B220$^+$ (Fig. 5*d*) cells. However, CM from T cell-reconstituted mice injected with IL-7 again showed an ≈4-fold increase in OC formation in both monocytes precursors (Fig. 5*b*) and B220$^+$ cells (Fig. 5*d*). In both athymic (−/−) and euthymic (+/−) mice, no significant difference in OC formation was observed between vehicle-treated mice and control wells treated with culture medium alone, confirming that unstimulated splenic T cells do not induce osteoclastogenesis.

To evaluate the contributions of RANKL and TNF-α, contained in the CMs, to OC formation, OCs were generated by using IL-7-stimulated CM (50% final volume) from euthymic (+/−) mice, and athymic (−/−) nude mice reconstituted with T cells, in the presence of a neutralizing antibody to TNF-α, OPG

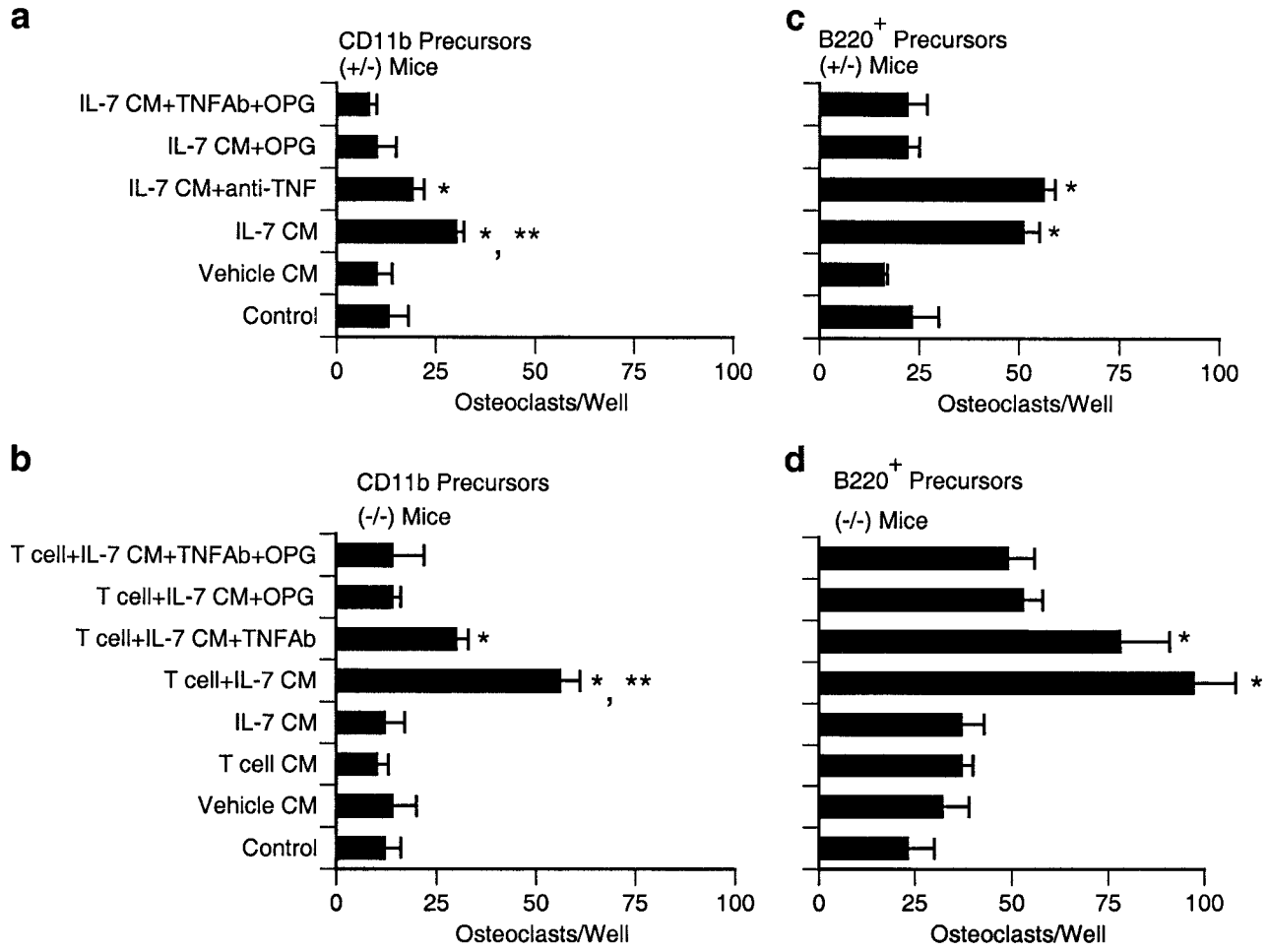

Fig. 5. CM from IL-7-stimulated T cell-replete mice stimulates OC formation by a mechanism involving RANKL and TNF-α. Purified OC precursors [CD11b (*a* and *b*) or B220$^+$IgM$^-$ cells (*c* and *d*)] were incubated in the presence of spleen cell CMs derived from euthymic (+/−) control mice (*a* and *c*) treated *in vivo* with vehicle or IL-7 or athymic (−/−) nude mice (*b* and *d*) treated *in vivo* with vehicle or reconstituted with T cells, with or without IL-7 administration. Additional wells treated with CM derived from *in vivo* IL-7-stimulated euthymic (+/−) spleen cells or athymic (−/−) *in vivo* IL-7-stimulated and T cell-reconstituted spleen cells, also received neutralizing TNF-α antibody, OPG, or both. The number of TRAP-positive multinucleated (≥3 nuclei) OCs formed was counted. Data show the average of triplicate cultures ± SEM and are representative of two independent experiments. *, $P < 0.001$ with respect to vehicle (ANOVA); **, $P < 0.001$ with respect to IL-7 CM + TNFAb for *a* and T cell + IL-7CM + TNFAb for *b* (ANOVA).

(the inhibitor of RANKL), or a combination of both. The data show (Fig. 5 *a* and *b*) that neutralizing antibody to TNF-α reduced by 35% and 45% for euthymic (+/−) control and athymic (−/−) nude mice, respectively, but did not completely abrogate OC formation induced by splenic supernatants when using monocytes as OC precursors. However, TNF-α neutralization had no significant effect on OC formation when using B220$^+$ cells as precursors (Fig. 5 *c* and *d*). However, the addition of OPG completely suppressed OCs formation induced by the CM to baseline levels, in both monocyte precursors and B220$^+$ cells. These data demonstrate that *in vivo* IL-7-stimulated splenic supernatants from mice replete in T cells [euthymic (+/−) control and T cell-reconstituted athymic (−/−) nude mice] induce OC formation by a mechanism involving RANKL. In the case of OC precursors derived from the monocyte lineage, RANKL-induced osteoclastogenesis is further augmented by TNF-α.

Discussion

Elevated levels of the osteoclastogenic cytokine IL-7 have long been associated with RA (2–4), an inflammatory disease characterized by local and systemic bone loss (5). Although IL-7 is known to induce bone loss *in vivo* (1), its mechanism of action remains poorly understood. Previous reports have suggested that IL-7 may act by elevating the number of bone marrow B220$^+$ cells, a population of early B cell precursors capable of differentiating into OCs under the action of osteoclastogenic cytokines (6). Based on *in vitro* observations, we previously suggested that T cells were a likely source of these osteoclastogenic cytokines (7). We now demonstrate *in vivo* that T cells are essential mediators of IL-7-induced bone loss. Our data show that, despite an up-regulation of B220$^+$ cells in the bone marrow, IL-7 does not induce bone loss *in vivo* in the absence of T cells. These results demonstrate that *in vivo* an enhanced supply of OC precursors (B220$^+$ cell expansion) is in and of itself insufficient to account for the bone loss induced by IL-7. Our data demonstrate that the presence of T cells is an absolute requirement to supply osteoclastogenic cytokines necessary to condition the differentiation of early OC precursors, be they B220$^+$ cells or monocytes, into mature bone resorbing OCs.

We show that IL-7 administered *in vivo* induces marked *ex vivo* RANKL production by splenocytes derived from T cell-replete but not T cell-deficient mice. Spleen cells from T cell-deficient mice failed to secrete enhanced RANKL concentrations in response to IL-7. It has been reported that, in an animal model of a RA, T cell-derived RANKL is central to the inflammatory

bone loss (8). However, the mechanism by which T cells are induced to produce RANKL remains unknown. Our data suggest that IL-7, a cytokine known to be up-regulated in RA (2–4), may be the key protagonist in inflammatory bone loss by inducing RANKL from T cells.

A small amount of OC formation was also observed in the control wells in the complete absence of CM and may be related either to small concentrations of osteoclastogenic cytokines in the fetal call serum or to precursors already exposed to osteoclastogenic cytokines *in vivo* before isolation.

The RANKL decoy receptor, OPG, a key regulator of RANKL activity and bone metabolism (18), was undetectable in spleen cell CM under all conditions tested. Because the RANKL ELISA specifically detects only active non-OPG bound RANKL, our data show that the concentration of free active RANKL secreted by T cells is substantially elevated by IL-7, regardless of whether IL-7 regulates OPG. However, it is conceivable that decreased OPG production could also account for some of the increased free RANKL detected.

In the case of monocyte-derived OC precursors, IL-7-induced TNF-α appears be an important augmenter of RANKL-mediated osteoclastogenesis. The failure of TNF-α to accentuate OC formation elicited from $B220^+$ precursors is consistent with the fact the $B220^+$ cells do not express TNF-α receptors. In contrast, monocytes that did respond to TNF-α are known to express the p55 TNF-α receptor, which is known to be important for RANKL synergism (9, 14).

Despite the lack of response to TNF-α, $B220^+$ cells generated approximately twice as many mature OCs as did cultures containing corresponding groups of purified monocytes. This difference may be the result of a high rate of $B220^+$ cell proliferation observed in culture, compared with monocytes, which are more differentiated and proliferate more slowly.

Because no significant difference in the magnitude of the bone loss between euthymic $(+/-)$ and athymic $(-/-)$ nude mice reconstituted with T cells was observed with both pQCT and DEXA, the data suggest that T cells mediate all of the observed effects of IL-7 on BMD. Our data thus demonstrate that T cells are essential and critical components of the mechanism by which IL-7 induced bone loss.

Because athymic $(-/-)$ nude mice show a spontaneous restoration of T cell populations during adult life (24), we used young (8-week-old) mice. Consequently, both pQCT and DEXA revealed an increase in bone density in vehicle-treated mice compared with baseline after 4 weeks. This phenomenon is related to the high rate of bone modeling that takes place in young mice. Interestingly, during the 30 days of the experiment, the euthymic $(+/-)$ control mice showed a more rapid increase in BMD than athymic $(-/-)$ nude mice. These results were consistent with lower baseline BMD values in nude mice compared with heterozygous littermates as well as with higher rates of baseline bone resorption in athymic $(-/-)$ nude mice as evidenced by an increased level of collagen C-terminal telopeptide secretion. Because no significant difference in OC formation was observed *in vitro* between vehicle-treated mice and control wells treated with culture medium alone, these data suggest that unstimulated splenic T cells do not contribute to baseline OC formation. However, it is possible that unstimulated T cells secrete factors inhibitory to bone resorption, thus explaining the low BMD and higher biochemical parameters of bone resorption observed in T cell-deficient nude mice. This hypothesis is consistent with published data suggesting that unactivated T cells can inhibit OC formation (25, 26). It is also possible that malfunction of other immune cells such as B cells and macrophages in nude mice stemming from the lack of T cells may contribute to aberrant baseline bone modeling.

Based on the cumulative data we propose a model whereby elevated levels of IL-7, such as occur under inflammatory conditions like RA, leads to bone loss by a dual mechanism. First, IL-7 elicits the T cell production of the key osteoclastogenic cytokines RANKL and TNF-α. Second, IL-7 leads to the expansion of the OC precursor pool by inducing the proliferation of bone marrow $B220^+$ cell populations (1, 6, 23). The cumulative effect of increased concentrations of circulating osteoclastogenic cytokines and enhanced numbers of early OC precursors (derived from both $B220^+$ IgM^- and monocytic populations), leads to the increased bone loss associated with inflammation.

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