DC-STAMP is essential for cell–cell fusion in osteoclasts and foreign body giant cells

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Osteoclasts are bone-resorbing cells that play a pivotal role in bone remodeling. Osteoclasts form large multinuclear giant cells by fusion of mononuclear osteoclasts. How cell fusion is mediated, however, is unclear. We identify the dendritic cell–specific transmembrane protein (DC-STAMP), a putative seven-transmembrane protein, by a DNA subtraction screen between multinuclear osteoclasts and mononuclear macrophages. DC-STAMP is highly expressed in osteoclasts but not in macrophages. DC-STAMP–deficient mice were generated, and osteoclast cell fusion was completely abrogated in homozygotes despite normal expression of osteoclast markers and cytoskeletal structure. As osteoclast multinucleation was restored by retroviral introduction of *DC-STAMP*, loss of cell fusion was directly attributable to a lack of DC-STAMP. Defects in osteoclast multinucleation reduce bone-resorbing activity, leading to osteopetrosis. Similar to osteoclasts, foreign body giant cell formation by macrophage cell fusion was also completely abrogated in DC-STAMP–deficient mice. We have thus identified an essential regulator of osteoclast and macrophage cell fusion, DC-STAMP, and an essential role of osteoclast multinucleation in bone homeostasis.

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Osteoclasts are unique bone-resorbing cells, and mice carrying a mutant gene responsible for osteoclast formation exhibit the osteopetrotic phenotype (1, 2). Foreign body giant cells (FBGCs), which are monocyte/macrophage lineage cells, are generated in response to foreign bodies at the site of implantation (3). Both osteoclasts and FBGCs form multinuclear cells by fusion of monocyte/macrophage lineage cells. Mononuclear macrophages recognize each other and fuse to form multinuclear giant cells; thus, cell surface molecules are considered to be fusion-mediating molecules in osteoclasts and macrophages. E-Cadherin and macrophage fusion receptor have been identified as fusion molecules for osteoclasts and macrophages through the use of neutralizing antibodies (4, 5). CD44, CD9, and CD81 are also candidates for cell fusion molecules; however, defects in multinucleation of osteoclasts and macrophages have not been identified by gene targeting (6, 7).

Dendritic cell–specific transmembrane protein (DC-STAMP) is a seven-transmembrane protein originally identified in dendritic cells or IL-4–stimulated macrophages (8, 9). Recently, it has been reported to induce differentiation of osteoclasts (10). However, the role of DC-STAMP in cell–cell fusion and in vivo bone formation remains unknown. We generated DC-STAMP–deficient mice, demonstrating that multinucleation of osteoclasts and macrophages is completely abrogated in *DC-STAMP*$^{-/-}$ mice.

RESULTS AND DISCUSSION

Identification and gene targeting of *DC-STAMP*

To identify molecules functioning in cell fusion, we undertook subtractive screening between multinuclear osteoclasts and mononuclear

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macrophages, both of which are derived from common precursor cells. Multinuclear osteoclasts were induced by macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL); macrophages, which do not fuse, were induced by M-CSF alone (11). The screen identified DC-STAMP (clone SU166), also known as IL-4–induced gene (9), which is specifically expressed in osteoclasts among cells of the monocyte/macrophage lineage (Fig. 1 A). Weak expression of DC-STAMP was detected in activated dendritic cells stimulated with granulocyte/M-CSF (GM-CSF) and RANKL (unpublished data), consistent with the fact that DC-STAMP was also cloned from a dendritic cell cDNA library (8). We identified two isoforms of *DC-STAMP* expressed at equivalent levels in osteoclasts: a full-length form (available from GenBank/EMBL/DDBJ under accession no. AY517483) and a splice variant lacking the third and fourth transmembrane domains and designated *DC-STAMP-splice* (available from GenBank/EMBL/DDBJ under accession no. AY517484; Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20050645/DC1).

GM-CSF is a potent inhibitor of osteoclastogenesis: cells treated with GM-CSF differentiate into immature dendritic cells that do not fuse even in the presence of M-CSF and RANKL (12). Expression of DC-STAMP was abolished in the presence of GM-CSF (Fig. 1 A), suggesting that DC-STAMP regulates cell fusion. To determine its function, we undertook gene targeting of *DC-STAMP* (Fig. 1 B and Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20050645/DC1). The ATG start codon of DC-STAMP is located in exon 2. The targeting vector was constructed to insert an *enhanced green fluorescent protein* (*EGFP*) sequence into exon 2 such that EGFP was in frame with amino acid Leu^{55} of DC-STAMP. This mutation resulted in deletion of the second through fifth transmembrane domains, resulting in mutation of both the full-length and splice variant form of DC-STAMP. Heterozygotes were mated, and DC-STAMP–nulls were generated at Mendelian ratios as determined at 4–8 wk of age ($^{-/-}$, 78 [23.3%]; $^{+/-}$, 179 [53.4%]; and $^{+/+}$, 78 [23.3%]). Targeted disruption of both the full-length and splice variant forms of *DC-STAMP* was confirmed by RT-PCR

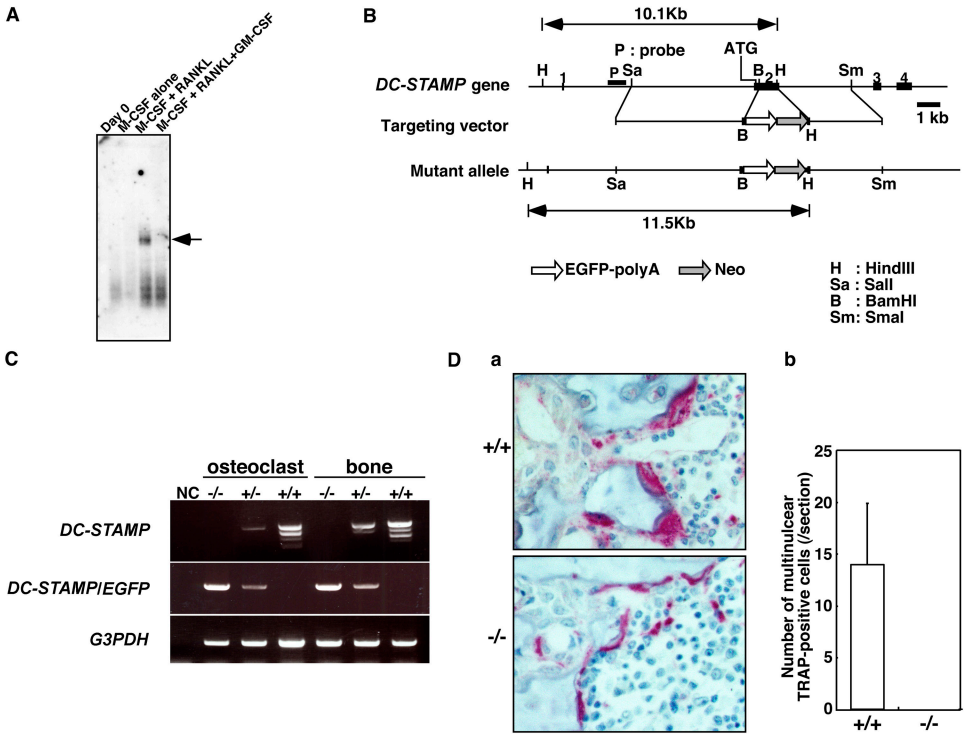

Figure 1. Identification and gene targeting of *DC-STAMP*. (A) Specific expression of *DC-STAMP* (arrow) in osteoclasts (M-CSF + RANKL) was determined among macrophages (M-CSF alone), immature dendritic cells (M-CSF + RANKL + GM-CSF), and osteoclasts derived from common precursor cells by Northern blot analysis. (B) The endogenous *DC-STAMP* locus (*DC-STAMP* gene), targeting vector, and targeted locus (mutant allele) are shown. Exons, represented by black boxes, are numbered. The ATG start codon is located in exon 2. The neomycin resistance gene (Neo) and *EGFP-polyA* sequence are indicated. The probe used for Southern hybridization is indicated (P). The *EGFP-polyA* sequence and Neo cassette were inserted into exon 2 to yield a construct encoding a DC-STAMP (1–55)–EGFP chimeric protein. (C) RT-PCR analysis of the expression of *DC-STAMP* and the chimeric *DC-STAMP-EGFP* genes in osteoclasts and bones from mice of the indicated genotypes. (D) TRAP staining in tibias of 8-wk-old *DC-STAMP*$^{+/+}$ or *DC-STAMP*$^{-/-}$ mice (a) and the number of multinuclear TRAP-positive cells (b). Multinuclear osteoclast formation was abrogated in *DC-STAMP*$^{-/-}$ mice. Values represent SD.

analysis using common primer sets to detect both forms (Fig. 1 C).

Multinucleation is abrogated in *DC-STAMP*$^{-/-}$ osteoclasts

Notably, no multinuclear osteoclasts were identified in bone sections of *DC-STAMP* homozygotes (Fig. 1 D). To confirm these observations, we performed in vitro culture of osteoclasts with an osteoclast marker. Tartrate-resistant acid phosphatase (TRAP)–positive multinuclear osteoclasts were generated from mononuclear cells derived from wild-type mice in the presence of M-CSF and RANKL, but multinucleation was completely abrogated in osteoclasts derived from *DC-STAMP*$^{-/-}$ mouse mononuclear cells (Fig. 2 A). Interestingly, mononuclear TRAP-positive cells were present in *DC-STAMP*$^{-/-}$ mice as shown in Fig. 2 A, suggesting that osteoclastogenesis occurs without cell fusion in *DC-STAMP*$^{-/-}$ osteoclasts. In fact, the total TRAP activity of TRAP-positive cells derived from *DC-STAMP*$^{-/-}$ mice was higher than that seen in wild-type mice (Fig. 2 B).

RANK signals play an important role in osteoclast differentiation, including multinucleation and bone resorption via up-regulating NFATc1 expression (13–15). The expression of osteoclast markers and transcription factors required for osteoclast differentiation, including RANK and NFATc1, was induced by M-CSF and RANKL in *DC-STAMP*$^{-/-}$ cells, as well as in wild-type cells (Fig. 2 C and Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20050645/DC1). Ruffled borders and actin rings, which are highly specific cytoskeletal features seen in osteoclasts, were formed in *DC-STAMP*$^{-/-}$ osteoclasts, in further support of the fact that DC-STAMP is not involved in mononuclear osteoclast development (Fig. 2 D and not depicted).

Multinuclear cell formation is dependent on cell density. The total number of nuclei in cultures of osteoclasts was comparable between *DC-STAMP*$^{-/-}$ and wild-type mice, indicating that inhibition of multinucleation was not caused by reduced cell density in *DC-STAMP*$^{-/-}$ cell cultures (Fig. 2 E). Furthermore, the proportion of osteoclast precursor cells (c-Fms^{+}c-Kit^{+}Mac1^{low}) in *DC-STAMP*$^{-/-}$ mice was equivalent to that seen in wild-type mice (Fig. 2 F), indicating that osteoclast differentiation from precursors to mononuclear osteoclasts was not affected. Osteoclasts derived from *DC-STAMP*$^{-/-}$ mice were mononuclear and exhibited bone-resorbing activity (Fig. 2 G); however, the bone-resorbing area exhibited in cultures of *DC-STAMP*$^{-/-}$ osteoclasts was small compared with wild-type cells (Fig. 2 G), even though the total number of nuclei was equal (Fig. 2 E), suggesting that multinucleation enhances the resorbing efficiency of osteoclasts.

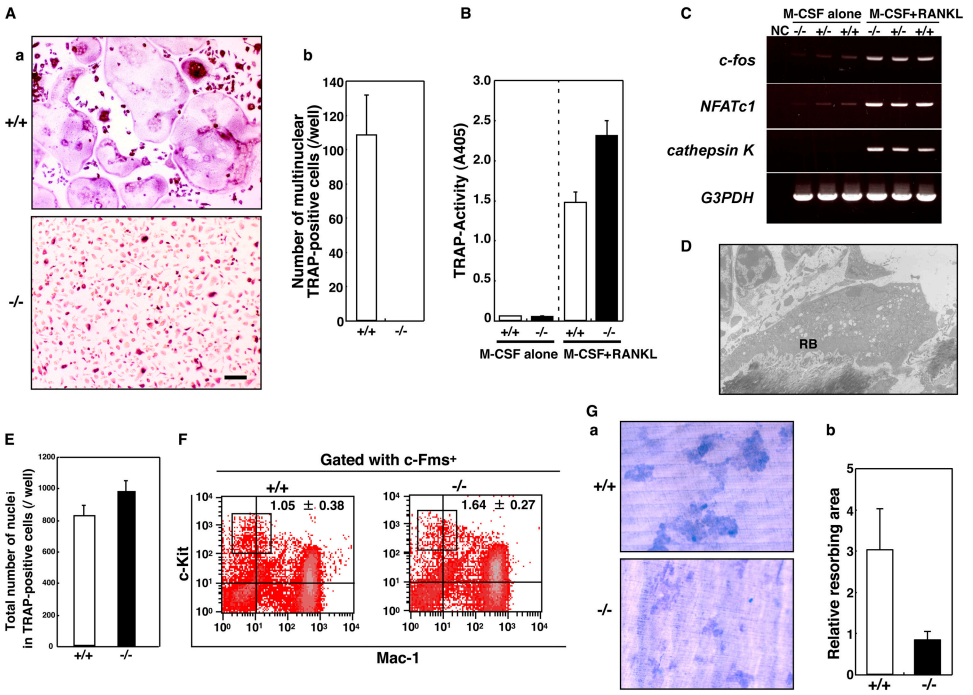

Figure 2. Lack of multinucleation in *DC-STAMP*$^{-/-}$ osteoclasts. (A) TRAP expression was induced, but multinucleation was completely abrogated in *DC-STAMP*$^{-/-}$ osteoclasts. TRAP staining (a) and the number of multinuclear TRAP-positive cells (b) are shown. Values represent SD. Bar, 50 μm. (B) TRAP solution assay of macrophages (M-CSF alone) and osteoclasts (M-CSF + RANKL). (C) Expression of *c-fos*, *NFATc1*, or *cathepsin K* in macrophages (M-CSF alone) or osteoclasts (M-CSF + RANKL) derived from *DC-STAMP*$^{+/+}$, *DC-STAMP*$^{+/-}$, or *DC-STAMP*$^{-/-}$ mice was analyzed by RT-PCR. NC, no template control. (D) Ruffled border formation was detected in osteoclasts of *DC-STAMP*$^{-/-}$ tibial sections under electron microscopy. RB, ruffled border. (E) Total number of nuclei in cultured osteoclasts. Values represent SD. (F) The percent frequency of a population of osteoclast precursor cells (boxes, c-Fms^{+}c-Kit^{+}Mac-1^{low}) is shown as the mean ± SD. (G) Resorbing lacunae were visualized by toluidine blue O staining (a), and relative resorbing areas were scored (b). Values represent SD.

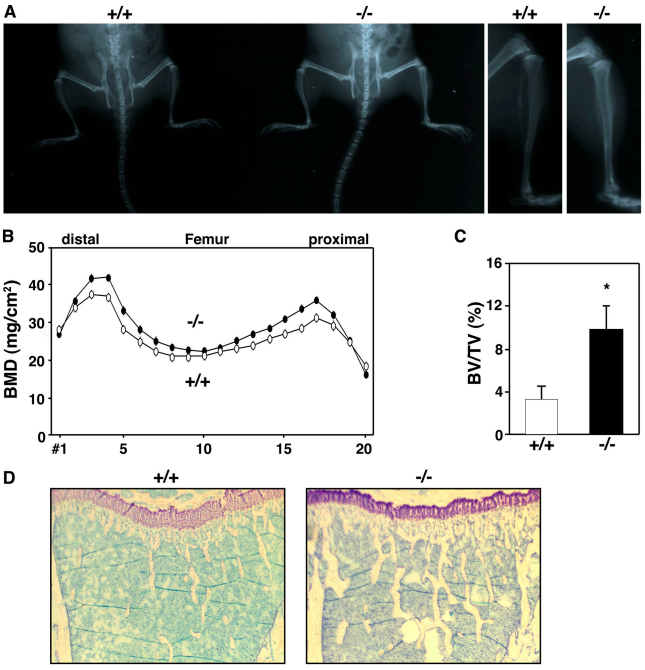

Figure 3. Increased bone mass in *DC-STAMP*$^{-/-}$ mice. (A) Soft x-ray analysis. Elevated radioopacity was observed in *DC-STAMP*$^{-/-}$ mice. (B) Increased BMD (mg/cm^2) was detected in *DC-STAMP*$^{-/-}$ mice (closed circles) compared with *DC-STAMP*$^{+/+}$ mice (open circles) by the dual energy x-ray absorptiometry method measured in 20 longitudinal divisions of femurs from 8-wk-old female mice. (C) Bone morphometric analysis of tibia from 8-wk-old mice. The percent BV/TV was elevated in *DC-STAMP*$^{-/-}$ mice compared with wild-type mice (*, $P < 0.01$). Values represent SD. (D) Increased numbers of trabeculae were observed in tibial sections of *DC-STAMP*$^{-/-}$ mice by toluidine blue staining.

Bone mass is increased in *DC-STAMP*$^{-/-}$ mice

Decreased bone resorption seen in *DC-STAMP*$^{-/-}$ compared with wild-type mice was observed as increased bone mass (Fig. 3). Soft x-ray analysis showed an elevated radioopacity in *DC-STAMP*$^{-/-}$ mice (Fig. 3 A). Bone mineral density (BMD) and bone volume per tissue volume (BV/TV) was increased in *DC-STAMP*$^{-/-}$ mice compared with wild-type mice (Fig. 3, B and C). Furthermore, trabecular bone mass was increased in *DC-STAMP*$^{-/-}$ mice (Fig. 3 D), indicating that loss of osteoclast cell fusion leads to an osteopetrotic phenotype. However, because tooth eruption and formation of the bone marrow cavity was normal in *DC-STAMP*$^{-/-}$ mice, such osteopetrosis was relatively mild compared with mouse mutants exhibiting strong osteopetrotic phenotypes such as c-Fos knockout or *op/op* mice (2, 16). Collectively, our observations indicate that bone-resorbing activity is stimulated by cell fusion and its disruption affects the bone as indicated by osteopetrosis.

DC-STAMP is indispensable for cell fusion of osteoclasts

To exclude the possibility that the lack of cell fusion seen in *DC-STAMP*$^{-/-}$ mice could be caused by reduced expression of other factors, we analyzed expression of factors known to function in cell fusion (4–6, 17). Such factors, which included macrophage fusion receptor, E-cadherin, meltrin-α, CD44, and CD47, were expressed similarly in *DC-STAMP*$^{-/-}$ as in wild-type mice (Fig. S4, available at http://www.jem.org/cgi/content/full/jem.20050645/DC1). Integrin $\alpha_v\beta_3$ plays a role in osteoclast adhesion and differentiation (18, 19); however, the expression level of adhesion molecules was unchanged in *DC-STAMP*$^{-/-}$ relative to wild-type osteoclasts (Fig. S5, available at http://www.jem.org/cgi/content/full/jem.20050645/DC1).

To confirm that DC-STAMP is indispensable for osteoclast fusion, *DC-STAMP* was transduced into osteoclast progenitor cells isolated from *DC-STAMP*$^{-/-}$ mice using retrovirus infection. This treatment effectively rescued osteoclast cell fusion, indicating that DC-STAMP is required for osteoclast cell fusion (Fig. 4 A). Transduction of the splice variant failed to induce cell fusion (Fig. 4 A). Currently, the function of that construct is unclear.

DC-STAMP is a member of the seven-transmembrane receptor family, and it may function as a chemokine receptor; if that were the case, loss of cell fusion could be caused by reduced cell contact caused by impaired cell migration. To test this hypothesis, cells were cultured at high density to induce migration-independent cell contact (18). Dramatic multinucleation was induced in osteoclasts derived from wild-type mice, whereas no fusion occurred in *DC-STAMP*$^{-/-}$ osteoclasts, indicating that fusion is inhibited even under cell-to-cell contact conditions (Fig. 4 B). Osteoblasts play a key role in osteoclast differentiation through cytokines such as M-CSF and RANKL or cell-adhesion signals. In the case of *DC-STAMP*$^{-/-}$ mice, the fusion signal was not rescued in vivo or by cocultivation with osteoblasts (Fig. 4 B). High doses of M-CSF have been reported to restore the osteoclastogenesis defect seen in *integrin* $\beta_3{}^{-/-}$ mice or *DAP12*$^{-/-}$ mice in vitro (19, 20). Long-term culture or high doses of M-CSF and RANKL did not induce cell fusion, and multinucleation was still completely abrogated in *DC-STAMP*$^{-/-}$ osteoclasts (unpublished data).

Next, we asked how cell fusion is mediated through DC-STAMP. Supernatants of wild-type osteoclast cultures did not induce fusion in *DC-STAMP*$^{-/-}$ osteoclasts, and supernatants from *DC-STAMP*$^{-/-}$ osteoclast cultures did not inhibit multinucleation of wild-type osteoclasts (unpublished data). Thus, cell fusion induced by DC-STAMP is not mediated via unknown soluble factors but by direct interaction between DC-STAMP and a putative ligand expressed by mononuclear osteoclasts. In that case, a homogeneous population of osteoclast precursors likely serves as founder and fusion-competent cells, similar to myoblasts. To address this possibility, mixed cultures of osteoclast precursors isolated from *DC-STAMP*$^{-/-}$ and wild-type mice were created. Interestingly, multinuclear EGFP-expressing cells were induced in these cultures in the presence of M-CSF plus RANKL, suggesting that fusion between EGFP-positive *DC-STAMP*$^{-/-}$ cells and wild-type osteoclast precursor cells was induced (Fig. 4 C). These data indicate two possibilities:

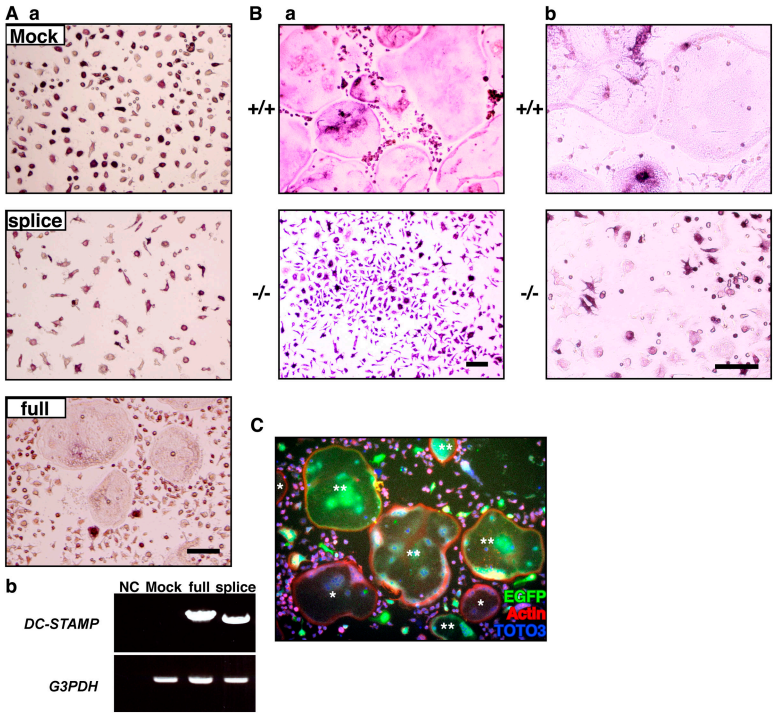

Figure 4. Rescue of cell fusion in *DC-STAMP*$^{-/-}$ osteoclasts. (A) Osteoclast precursors from *DC-STAMP*$^{-/-}$ mice were transduced by retrovirus expressing full-length (full) or a splice variant form (splice) of *DC-STAMP* (a). The indicated form of *DC-STAMP* expressed via retroviral infection was detected in *DC-STAMP*$^{-/-}$ osteoclasts by RT-PCR analysis (b). NC, no template control. Bar, 50 μm. (B) No multinuclear TRAP-positive cells were induced in *DC-STAMP*$^{-/-}$ cells by high density culture (a) or cocultivation with osteoblasts (b). Bar, 50 μm. (C) 2×10^4 cells/well of M-CSF–dependent osteoclast precursors from *DC-STAMP*$^{-/-}$ and *DC-STAMP*$^{+/+}$ mice were mixed and cultured in the presence of M-CSF and RANKL for 4 d in 96-well culture plates. Cells were stained by rabbit anti-EGFP antibody followed by Alexa 488 (EGFP)–conjugated anti-rabbit IgG antibody with rhodamine-conjugated phalloidin for F-actin staining and TOTO3 for nuclear staining. Note the formation of EGFP-positive multinuclear cells. *, EGFP-negative multinuclear cells; **, EGFP-positive multinuclear cells.

(a) a putative DC-STAMP ligand is expressed on osteoclasts, or (b) expression of DC-STAMP is required only in fusion founder cells and not in both founder and fusion-competent cells. Heterogeneous EGFP expression in DC-STAMP–EGFP knock-in cells during the course of osteoclast differentiation (unpublished data) supports the latter possibility. Thus, DC-STAMP might be involved in cell–cell interactions in a receptor–ligand fashion.

Multinucleation is abrogated in *DC-STAMP*$^{-/-}$ FBGCs

DC-STAMP may also be required to form other monocyte-derived multinucleated giant cells, such as FBGCs. FBGCs are induced by fusion of macrophages in response to foreign bodies at the site of implantation (3). Interestingly, multinucleation of FBGCs induced by implantation of foreign bodies was also inhibited in *DC-STAMP*$^{-/-}$ mice (Fig. 5 A). FBGCs are formed in vitro from monocyte progenitors by stimulation with cytokines such as IL-3 and IL-4 (21). FBGC formation by combined IL-3 and IL-4 treatment was abrogated in *DC-STAMP*$^{-/-}$ cells (Fig. 5 B), indicating that DC-STAMP is universally required for monocyte lineage cell fusion. The evidence that DC-STAMP is overexpressed in giant cell tumors, which are primary bone neoplasm-containing, multinucleated osteoclast-like giant cells compared with normal tissues (22), supports involvement of DC-STAMP in pathological giant cell formation.

DC-STAMP was originally isolated from dendritic cells, which do not fuse (8). We are now evaluating the function of dendritic cells in DC-STAMP–deficient mice. Dendritic cells differentiate from the same precursors as osteoclasts, and stimulation of dendritic cell differentiation inhibits osteoclast formation, including multinucleation (12). These findings suggest that expression of a putative DC-STAMP ligand might require osteoclast induction stimuli, or that intracellular events after DC-STAMP binding to such a ligand in osteoclasts play an important role in cell fusion. DC-STAMP has been reported to be expressed on the surface of osteoclasts (10) and in the endoplasmic reticulum of *DC-STAMP*–transduced dendritic cells or 293 cells (23). Phagosomes fuse with the endoplasmic reticulum in dendritic cells (24), suggesting that DC-STAMP may function in such a cytoplasmic membrane fusion. Thus, differences in DC-STAMP localization may explain differences of multinucleation between osteoclasts/FBGCs and dendritic cells, both of which express DC-STAMP. Identification of a DC-STAMP ligand should resolve these questions.

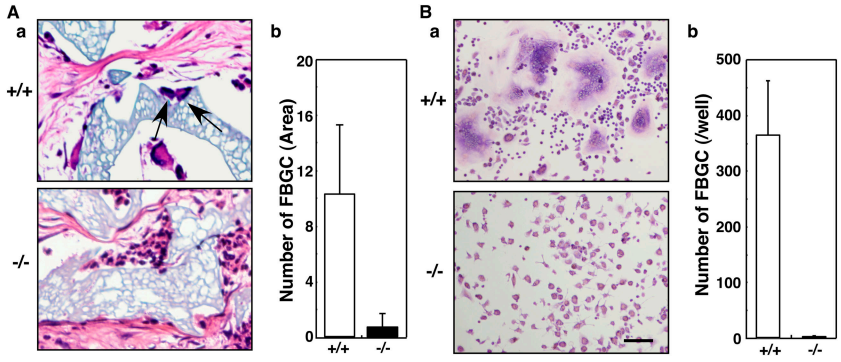

Figure 5. Macrophage cell fusion is abrogated in *DC-STAMP*$^{-/-}$ mice. (A) Histological analysis of implants in *DC-STAMP*$^{-/-}$ and *DC-STAMP*$^{+/+}$ mice. Hematoxylin and eosin staining (a) and the number of FBGCs (b) are shown. Arrows indicate FBGCs. (B) FBGCs were induced and stained with May-Gruenwald Giemsa (a), and the number of FBGCs containing more than three nuclei was scored (b). Values represent SD. Bar, 50 μm.

MATERIALS AND METHODS

Targeted disruption of the *DC-STAMP* gene. The *EGFP-polyA* gene was inserted into the BamHI site in exon 2 in frame with the juxtamembrane region of the first transmembrane domain of *DC-STAMP*. Exon 2 was replaced by *EGFP-polyA* and the *PGK-Neo* gene to yield a targeting vector. The Ethics Review Committee for Animal Experimentation of Keio University approved the experimental protocol.

Analysis of skeletal morphology. BMD (mg/cm^2) and bone radiographs of 8-wk-old *DC-STAMP*$^{+/+}$ and *DC-STAMP*$^{-/-}$ littermates were measured by the dual energy x-ray absorptiometry method using a DCS-600R (Aloka Co. Ltd.) and a soft x-ray apparatus (Softex Co. Ltd.), respectively. BV/TV was determined by bone morphometric analysis.

Cell culture. Macrophages and osteoclasts were induced in the presence of M-CSF and RANKL or by cocultivation of osteoclast precursor cells with osteoblasts as previously described (11). Osteoclastogenesis was evaluated by either TRAP staining or a TRAP solution assay as previously described (18). For the pit formation assay, retroviral transduction, and FBGC formation assay, unfractionated BM cells were pretreated with 50 ng/ml M-CSF for 2 d, and M-CSF–dependent cells were harvested and used as progenitor cells. The resorbing lacunae were visualized by toluidine blue O staining (15), and the relative resorbing area was scored under a microscope (IX70; Olympus). Preparation of retrovirus was as previously described (12). For FBGC induction, M-CSF–dependent progenitors were cultured in the presence of 100 ng/ml IL-3 (Wako) and 100 ng/ml IL-4 (R&D Systems) for 4 d, and May-Gruenwald Giemsa staining was performed.

Immunohistochemical staining. Cells cultured in dishes were fixed with 4% paraformaldehyde/PBS and stained with the anti-EGFP antibody (Mo Bio Laboratories, Inc.), followed by Alexa 488–conjugated anti–rabbit IgG antibody (Invitrogen) with TOTO3 (Invitrogen) for nuclear staining, and F-actin was stained by rhodamine-conjugated phalloidin (Invitrogen). For electron microscopic analysis, ultrathin sections were stained with uranyl acetate and lead citrate and observed with an electron microscope (H-7500; Hitachi).

In vivo FBGC formation. Ivalon surgical product (M-PACT, 10 × 10 × 0.5 mm; Eudora) was implanted s.c. in *DC-STAMP*$^{-/-}$ and *DC-STAMP*$^{+/+}$ mice. After 12 d, implants were harvested, and histological analyses were performed by hematoxylin and eosin staining. Multinuclear cells containing more than three nuclei that adhered to implants were scored as FBGC in five independent fields per section isolated from four independent implanted mice.

RT-PCR analysis. Total RNA was extracted from cultured macrophages or osteoclasts using an RNeasy mini kit (QIAGEN). First-strand cDNA was prepared, and PCR was performed as previously described (18). Primer sets used to detect various molecules are as previously described (12, 15, 18).

Online supplemental material. Figs. S1 and S2 describe the amino acid sequence information of DC-STAMP and the generation of DC-STAMP/EGFP knock-in mice, respectively. Fig. S3 shows the expression of transcription factors, Fig. S4 depicts candidate molecules for cell fusion, and Fig. S5 shows adhesion molecules in DC-STAMP$^{-/-}$, $^{+/-}$, or $^{+/+}$ osteoclasts. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20050645/DC1.

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REFERENCES

1. Boyce, B.F., T. Yoneda, C. Lowe, P. Soriano, and G.R. Mundy. 1992. Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. *J. Clin. Invest.* 90:1622–1627.
2. Grigoriadis, A.E., Z.Q. Wang, M.G. Cecchini, W. Hofstetter, R. Felix, H.A. Fleisch, and E.F. Wagner. 1994. c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. *Science.* 266:443–448.
3. Kyriakides, T.R., M.J. Foster, G.E. Keeney, A. Tsai, C.M. Giachelli, I. Clark-Lewis, B.J. Rollins, and P. Bornstein. 2004. The CC chemokine ligand, CCL2/MCP1, participates in macrophage fusion and foreign body giant cell formation. *Am. J. Pathol.* 165:2157–2166.
4. Mbalaviele, G., H. Chen, B.F. Boyce, G.R. Mundy, and T. Yoneda. 1995. The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow. *J. Clin. Invest.* 95:2757–2765.
5. Saginario, C., H. Sterling, C. Beckers, R. Kobayashi, M. Solimena, E. Ullu, and A. Vignery. 1998. MFR, a putative receptor mediating the fusion of macrophages. *Mol. Cell. Biol.* 18:6213–6223.
6. de Vries, T.J., T. Schoenmaker, W. Beertsen, R. van der Neut, and V. Everts. 2005. Effect of CD44 deficiency on in vitro and in vivo osteoclast formation. *J. Cell. Biochem.* 94:954–966.
7. Takeda, Y., I. Tachibana, K. Miyado, M. Kobayashi, T. Miyazaki, T. Funakoshi, H. Kimura, H. Yamane, Y. Saito, and H. Goto. 2003. Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. *J. Cell Biol.* 161:945–956.
8. Hartgers, F.C., J.L. Vissers, M.W. Looman, C. van Zoelen, C. Huf-

fine, C.G. Figdor, and G.J. Adema. 2000. DC-STAMP, a novel multimembrane-spanning molecule preferentially expressed by dendritic cells. *Eur. J. Immunol.* 30:3585–3590.
9. Staege, H., A. Brauchlin, G. Schoedon, and A. Schaffner. 2001. Two novel genes FIND and LIND differentially expressed in deactivated and *Listeria*-infected human macrophages. *Immunogenetics*. 53:105–113.
10. Kukita, T., N. Wada, A. Kukita, T. Kakimoto, F. Sandra, K. Toh, K. Nagata, T. Iijima, M. Horiuchi, H. Matsusaki, et al. 2004. RANKL-induced DC-STAMP is essential for osteoclastogenesis. *J. Exp. Med.* 200:941–946.
11. Arai, F., T. Miyamoto, O. Ohneda, T. Inada, T. Sudo, K. Brasel, T. Miyata, and D.M. Anderson. 1999. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor κB (RANK) receptors. *J. Exp. Med.* 190:1741–1754.
12. Miyamoto, T., O. Ohneda, F. Arai, K. Iwamoto, S. Okada, K. Takagi, D.M. Anderson, and T. Suda. 2001. Bifurcation of osteoclasts and dendritic cells from common progenitors. *Blood*. 98:2544–2554.
13. Takayanagi, H., S. Kim, T. Koga, H. Nishina, M. Isshiki, H. Yoshida, A. Saiura, M. Isobe, T. Yokochi, J. Inoue, et al. 2002. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev. Cell.* 3:889–901.
14. Matsuo, K., D.L. Galson, C. Zhao, L. Peng, C. Laplace, K.Z. Wang, M.A. Bachler, H. Amano, H. Aburatani, H. Ishikawa, et al. 2004. Nuclear factor of activated T-cells (NFAT) rescues osteoclastogenesis in precursors lacking c-Fos. *J. Biol. Chem.* 279:26475–26480.
15. Iwamoto, K., T. Miyamoto, Y. Sawatani, N. Hosogane, I. Hamaguchi, M. Takami, K. Nomiyama, K. Takagi, and T. Suda. 2004. Dimer formation of receptor activator of nuclear factor kappaB induces incomplete osteoclast formation. *Biochem. Biophys. Res. Commun.* 325:229–234.
16. Yoshida, H., S. Hayashi, T. Kunisada, M. Ogawa, S. Nishikawa, H. Okamura, T. Sudo, L.D. Shultz, and S. Nishikawa. 1990. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature*. 345:442–444.
17. Han, X., H. Sterling, Y. Chen, C. Saginario, E.J. Brown, W.A. Frazier, F.P. Lindberg, and A. Vignery. 2000. CD47, a ligand for the macrophage fusion receptor, participates in macrophage multinucleation. *J. Biol. Chem.* 275:37984–37992.
18. Miyamoto, T., F. Arai, O. Ohneda, K. Takagi, D.M. Anderson, and T. Suda. 2000. An adherent condition is required for formation of multinuclear osteoclasts in the presence of macrophage colony-stimulating factor and receptor activator of nuclear factor kappa B ligand. *Blood*. 96:4335–4343.
19. Faccio, R., S. Takeshita, A. Zallone, F.P. Ross, and S.L. Teitelbaum. 2003. c-Fms and the alphavbeta3 integrin collaborate during osteoclast differentiation. *J. Clin. Invest.* 111:749–758.
20. Faccio, R., W. Zou, G. Colaianni, S.L. Teitelbaum, and F.P. Ross. 2003. High dose M-CSF partially rescues the Dap12−/− osteoclast phenotype. *J. Cell. Biochem.* 90:871–873.
21. McNally, A.K., and J.M. Anderson. 1995. Interleukin-4 induces foreign body giant cells from human monocytes/macrophages. Differential lymphokine regulation of macrophage fusion leads to morphological variants of multinucleated giant cells. *Am. J. Pathol.* 147:1487–1499.
22. Skubitz, K.M., E.Y. Cheng, D.R. Clohisy, R.C. Thompson, and A.P. Skubitz. 2004. Gene expression in giant-cell tumors. *J. Lab. Clin. Med.* 144:193–200.
23. Eleveld-Trancikova, D., V. Triantis, V. Moulin, M.W. Looman, M. Wijers, J.A. Fransen, A.A. Lemckert, M.J. Havenga, C.G. Figdor, R.A. Janssen, et al. 2004. The dendritic cell-derived protein DC-STAMP is highly conserved and localizes to the endoplasmic reticulum. *J. Leukoc. Biol.* 77:337–343.
24. Guermonprez, P., L. Saveanu, M. Kleijmeer, J. Davoust, P. Van Endert, and S. Amigorena. 2003. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature*. 425:397–402.