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An Essential Role for STAT6-STAT1 Protein Signaling in Promoting Macrophage Cell-Cell Fusion[S]

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Background: The signaling leading to macrophage fusion remains largely unknown.
Results: STAT6 deficiency completely inhibited macrophage fusion, although STAT1 deficiency or OC-STAMP/DC-STAMP co-expression was sufficient to promote macrophage fusion.
Conclusion: The STAT6-STAT1-OC-STAMP/DC-STAMP axis is required for macrophage fusion.
Significance: The STAT6-STAT1-OC-STAMP/DC-STAMP axis is a novel pathway leading to macrophage fusion.

Macrophage lineage cells such as osteoclasts and foreign body giant cells (FBGCs) form multinuclear cells by cell-cell fusion of mononuclear cells. Recently, we reported that two seven-transmembrane molecules, osteoclast stimulatory transmembrane protein (OC-STAMP) and dendritic cell-specific transmembrane protein (DC-STAMP), were essential for osteoclast and FBGC cell-cell fusion *in vivo* and *in vitro*. However, signaling required to regulate FBGC fusion remained largely unknown. Here, we show that signal transducer and activator of transcription 1 (STAT1) deficiency in macrophages enhanced cell-cell fusion and elevated *DC-STAMP* expression in FBGCs. By contrast, lack of STAT6 increased STAT1 activation, significantly inhibiting cell-cell fusion and decreasing *OC-STAMP* and *DC-STAMP* expression in IL-4-induced FBGCs. Furthermore, either STAT1 loss or co-expression of OC-STAMP/DC-STAMP was sufficient to induce cell-cell fusion of FBGCs without IL-4. We conclude that the STAT6-STAT1 axis regulates *OC-STAMP* and *DC-STAMP* expression and governs fusogenic mechanisms in FBGCs.

Multinuclear cells derived from hematopoietic stem cells or monocyte/macrophage lineage cells consist of osteoclasts, bone resorbing cells, and macrophage giant cells, which include foreign body giant cells (FBGCs).[3] FBGCs are induced at sites of implanted biomaterials and tumors, as well as in chronic inflammation or infection such as tuberculosis. Both FBGCs and osteoclasts emerge from common progenitors and undergo multinucleation, which was first demonstrated by cell-cell fusion of mononuclear cells (1). The function of FBGC cell-cell fusion is likely removal or degradation of foreign materials (2, 3). FBGCs form in response to Th2 cytokines such as IL-4 and IL-13 (4–6), and signal transducer and activator of transcription factor (STAT)-6 plays an essential role in transducing IL-4/IL-4R signals. However, mechanisms underlying FBGC cell-cell fusion remain largely unknown.

To date, various molecules have been identified as regulating macrophage and/or osteoclast cell-cell fusion. Macrophage fusion receptor (MFR; also called SHPS-1), a member of immunoglobulin (Ig) superfamily, was shown to function in macrophage cell-cell fusion based on loss-of-function assays using MFR monoclonal antibodies or the soluble form of the extracellular domain of MFR (7, 8). CD47, an MFR ligand, and the intracellular domain of CD44 (CD44ICD) have also been implicated in macrophage cell-cell fusion (9, 10). DAP12, an ITAM motif-containing adaptor protein, and the signaling molecule Syk were also reportedly involved in cell-cell fusion of macrophages (11). Furthermore, studies employing antisense oligonucleotides and knock-out mice showed that Meltrin α (also called ADAM12) and matrix metalloprotease 9 (MMP9) were involved in multinucleation of macrophages and osteoclasts, respectively (12, 13). Defective fusion of macrophages and osteoclasts was also demonstrated in MCP-1/CCL2 or ATP6v0d2 knock-out cells (14, 15). In studies of osteoclasts employing neutralizing antibody and knock-out mice, respectively, E-cadherin and CD200 were reported to play a role in cell-cell fusion (16, 17). Recently, two multimembrane-spanning molecules, dendritic cell-specific transmembrane protein (DC-STAMP) and osteoclast stimulatory transmembrane protein (OC-STAMP), were identified as required for cell-cell fusion of both macrophages and osteoclasts by generating knock-out mice (18, 19).

Here, we employ STAT1-deficient and STAT6-deficient mice to show that STAT6 activation by IL-4 inhibits STAT1,

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[3] The abbreviations used are: FBGC, foreign body giant cell; MFR, macrophage fusion receptor; DC-STAMP, dendritic cell-specific transmembrane protein; OC-STAMP, osteoclast stimulatory transmembrane protein; BMM, bone marrow macrophage; Tg, transgenic; RANKL, receptor activator of NF-κB ligand; MFR, macrophage fusion receptor.

leading to expression of OC-STAMP and DC-STAMP in macrophages. STAT1 deficiency or co-expression of OC-STAMP and DC-STAMP was sufficient to promote macrophage cell-cell fusion without IL-4. These observations define molecular mechanisms underlying cell-cell fusion of macrophages.

EXPERIMENTAL PROCEDURES

Mice—STAT1-deficient and STAT6-deficient mice were purchased from Taconic (Hudson, NY) and The Jackson Laboratory (Bar Harbor, ME), respectively. OC-STAMP Tg mice were generated as described previously (19). Animals were maintained under specific pathogen-free conditions in animal facilities certified by the Keio University School of Medicine Animal Care Committee. Animal protocols were approved by that committee.

Reagents—M-CSF, RANKL, GM-CSF, and IL-4 were purchased from R&D Systems Inc. (Minneapolis, MN). FK506 was purchased from Astellas Pharma (Tokyo, Japan).

In Vitro Osteoclastogenesis and Foreign Body Giant Cell Formation—Cell culture and tartrate resistance acid phosphatase and May-Grünwald Giemsa staining were performed as described previously (18, 20–23). Briefly, BM cells were cultured in the presence of M-CSF (50 ng/ml) for 3 days and then harvested as common progenitors of osteoclasts and FBGCs. Progenitors were cultured in the presence of M-CSF alone (50 ng/ml) for macrophages, M-CSF (50 ng/ml) + RANKL (25 ng/ml) for osteoclasts, GM-CSF alone (50 ng/ml) for immature dendritic cells, and GM-CSF (50 ng/ml) + IL-4 (50 ng/ml) for FBGCs.

FBGC Formation in Vivo—A PVA sponge was implanted subcutaneously in WT, STAT6-deficient, and STAT1-deficient mice. After 3 days, implants were harvested, and histological analysis was performed using hematoxylin and eosin staining.

Real Time PCR and RT-PCR Analysis—β-Actin and GAPDH expression served as internal controls for real time PCR and RT-PCR, respectively. Primer sequences were as follows for real time PCR: β-actin forward, 5′-TGAGAGGGAAATCGTGCGTGAC-3′, and β-actin reverse, 5′-AAGAAGGAAGGCTGGAAAAGAG-3′; OC-STAMP forward, 5′-ATGAGGACCATCAGGGCAGCCACG, and OC-STAMP reverse, 5′-GGAGAAGCTGGGTCAGTAGTTCGT; DC-STAMP forward, 5′-TCCTCCATGAACAAACAGTTCCAA-3′, and DC-STAMP reverse, 5′-AGACGTGGTTTAGGAATGCAGCTC-3′; NFATc1 forward, 5′-CAAGTCCTCACCACAGGGCTCACTA-3′, and NFATc1 reverse; 5′-GCGTGAGAGAGGTTCATTCTCCAAGT-3′; for RT-PCR: GAPDH forward, 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′, and GAPDH reverse, 5′-CATGTAGGCCATGAGGTCCACCAC-3′; OC-STAMP forward, 5′-TCACTGACCTGCGTTTCGACAA-3′, and OC-STAMP reverse, 5′-GCGTAGGCCTGTAGCCACCAA-3′; and DC-STAMP forward, 5′-ACTAGAGGAGAAGTCCTGGGAGTC-3′, and DC-STAMP reverse, 5′-CACCCACATGTAGAGATAGGTCAG-3′.

Western Blot Analysis—Whole-cell lysates were prepared from BM cultures, and equivalent amounts of protein were separated by SDS-PAGE and transferred to a PVDF membrane (Millipore) (23). Proteins were detected using the following antibodies: anti-STAT1, anti-phosphorylated STAT1 (Ser-727), and anti-STAT6 were purchased from Cell Signaling Technology (Beverly, MA); anti-phosphorylated STAT6 was from Abcam (Cambridge, MA), and anti-actin was from Sigma. STAT1 and pSTAT1 bands were quantified using National Institutes of Health imaging.

Statistical Analysis—p values were calculated using the unpaired Student's *t* test.

RESULTS

OC-STAMP Expression in FBGCs Requires STAT6—DC-STAMP and OC-STAMP are required for cell-cell fusion of macrophage lineage cells (18, 19), and first we analyzed the expression of these molecules in bone marrow macrophages (BMMs) treated with M-CSF, GM-CSF, or GM-CSF plus IL-4 *in vitro* (Fig. 1). *DC-STAMP* is reportedly expressed in GM-CSF-induced dendritic cells (24) but not in M-CSF-induced mononuclear macrophages (18). Indeed, *DC-STAMP* expression was significantly higher in GM-CSF-treated compared with M-CSF-treated BMMs and was further up-regulated by IL-4 in addition to GM-CSF, a condition known to induce FBGCs (Fig. 1*A*). By contrast, although GM-CSF alone did not induce *OC-STAMP* expression in BMMs, *OC-STAMP* expression was significantly up-regulated by treatment with GM-CSF plus IL-4 (Fig. 1*A*). IL-4 signaling is transduced via STAT6 (25–27), suggesting that *OC-STAMP* expression requires STAT6. We did not detect STAT6 phosphorylation in M-CSF- or GM-CSF-treated BMMs, but we observed strong STAT6 phosphorylation in GM-CSF plus IL-4-treated FBGCs (Fig. 1*B*). Thus, we analyzed FBGC formation in STAT6-deficient mice and found that multinuclear FBGC formation was abrogated *in vitro* and *in vivo* (Fig. 1, *C* and *D*). *DC-STAMP* and *OC-STAMP* expression, as analyzed by RT-PCR and real time PCR, were both significantly inhibited in STAT6-deficient FBGCs (Fig. 1, *E* and *F*), strongly suggesting that both are STAT6 targets. However, although *DC-STAMP* expression was significantly inhibited in STAT6-deficient FBGCs, residual *DC-STAMP* expression remained (Fig. 1, *E* and *F*), suggesting that *DC-STAMP* expression is regulated in part by a STAT6-independent mechanism. MMP9, a marker of differentiated FBGCs (13, 28), was expressed in STAT6-deficient cells at levels equivalent to those seen in wild-type cells (supplemental Fig. S1*A*), suggesting that DC-STAMP and OC-STAMP expression rather than differentiation was specifically inhibited in STAT6-deficient cells. Indeed, STAT6-deficient macrophages exhibited phagocytotic activity equivalent to that seen in wild-type cells (supplemental Fig. S1*B*).

OC-STAMP Expression Is Regulated by Different Mechanisms in FBGCs and Osteoclasts—In contrast to FBGCs, STAT6-deficient osteoclasts showed moderate stimulation rather than inhibition of multinucleation (Fig. 2*A*). *OC-STAMP* expression was equivalent in both STAT6-deficient and wild-type osteoclasts (Fig. 2*B*), suggesting that its expression in osteoclasts is STAT6-independent. Indeed, we found that STAT6 was not activated in osteoclasts (Fig. 2*C*). In addition, we recently found that *OC-STAMP* expression in osteoclasts is regulated directly by NFATc1, an transcription factor essential for osteoclastogenesis, and that osteoclast multinucleation and

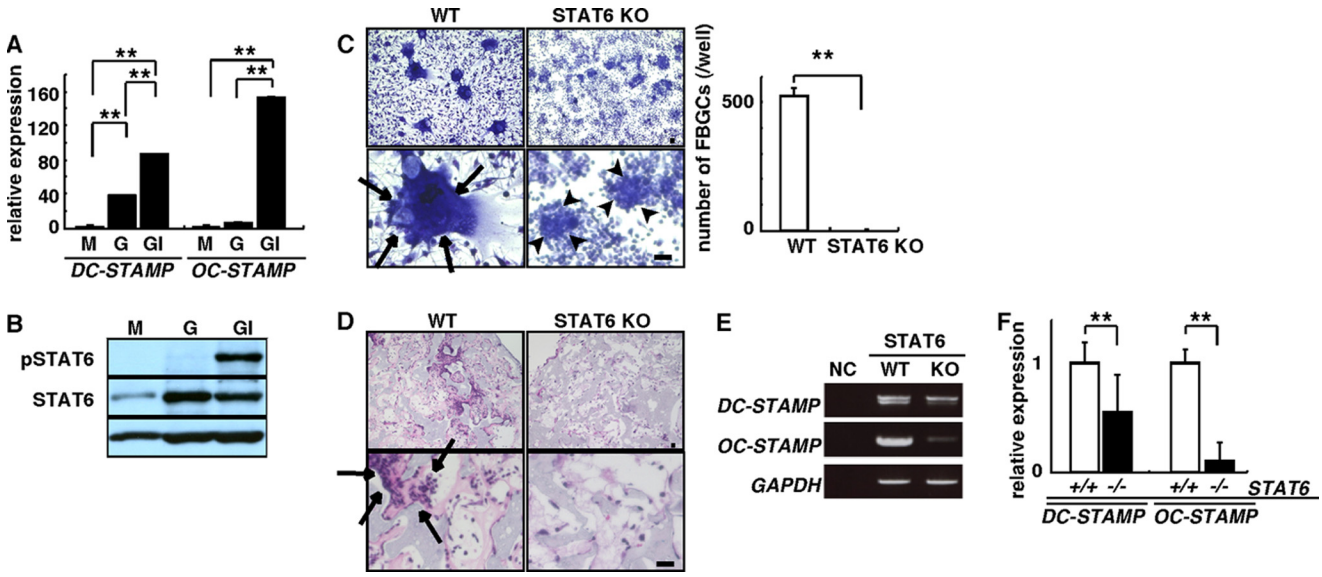

FIGURE 1. **STAT6 regulates both cell-cell fusion and *DC-STAMP/OC-STAMP* expression in FBGCs.** *A,* BMMs isolated from wild-type mice were cultured in the presence of M-CSF (*M*), GM-CSF (*G*), or GM-CSF + IL-4 (*GI*) for 6 days, and *DC-STAMP* and *OC-STAMP* expression was analyzed by real time PCR. **, $p < 0.001$. *B,* BMMs isolated from wild-type mice were cultured in the presence of M-CSF (*M*), GM-CSF (*G*), or GM-CSF + IL-4 (*GI*) for 6 days, and STAT6 phosphorylation was analyzed by Western blot. *C,* BMMs were isolated from wild-type (*WT*) and STAT6-deficient (*STAT6 KO*) mice and cultured in the presence of GM-CSF + IL-4 for 6 days. Cells were then stained with May-Grünwald Giemsa (*left panels*, *top*, low magnification; *bottom*, higher magnification), and the number of multinuclear FBGCs (more than three nuclei) was scored (*right panel*). *Arrows* and *arrowheads* indicate fused cells and aggregated cells, respectively. **, $p < 0.001$. *Bar,* 50 μm. *D,* PVA sponges were implanted subcutaneously in WT and STAT6 KO mice. Three days after implantation, sponges were stained with hematoxylin and eosin (*top*, low magnification; *bottom*, higher magnification). Fused cells are indicated by *arrows*. *Bar,* 50 μm. *E, DC-STAMP* and *OC-STAMP* expression in FBGCs derived from WT and STAT6-deficient mice (KO) was analyzed by RT-PCR. *GAPDH* served as an internal control. *NC,* no template control. *F, DC-STAMP* and *OC-STAMP* expression in FBGCs derived from wild-type (+/+) or STAT6-KO(−/−) mice was analyzed by real time PCR. **, $p < 0.001$.

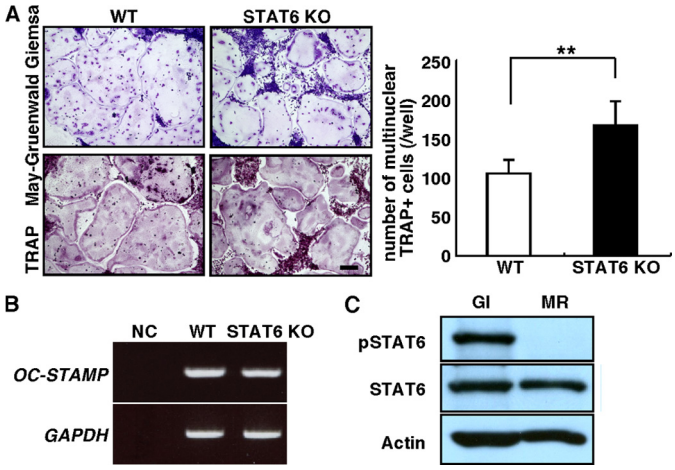

FIGURE 2. **STAT6 is dispensable for *OC-STAMP* expression in osteoclasts.** *A,* BMMs were isolated from wild-type or STAT6 KO mice and cultured in the presence of M-CSF plus RANKL for 6 days. Cells were then stained with May-Grünwald Giemsa and tartrate resistance acid phosphatase (*TRAP*) (*left panel*), and the number of multinuclear osteoclasts containing more than three nuclei was scored (*right panel*). **, $p < 0.001$. *Bar,* 50 μm. *B, OC-STAMP* expression in osteoclasts derived from wild-type (*WT*) or STAT6 KO mice was analyzed by RT-PCR. *GAPDH* served as internal control. *NC,* no template control. *C,* STAT6 phosphorylation, total STAT6 protein and actin levels were analyzed by Western blot in cells treated with M-CSF (50 ng/ml) + RANKL (25 ng/ml) (*MR*, osteoclasts) and GM-CSF (50 ng/ml) plus IL-4 (50 ng/ml) (*GI,* FBGCs).

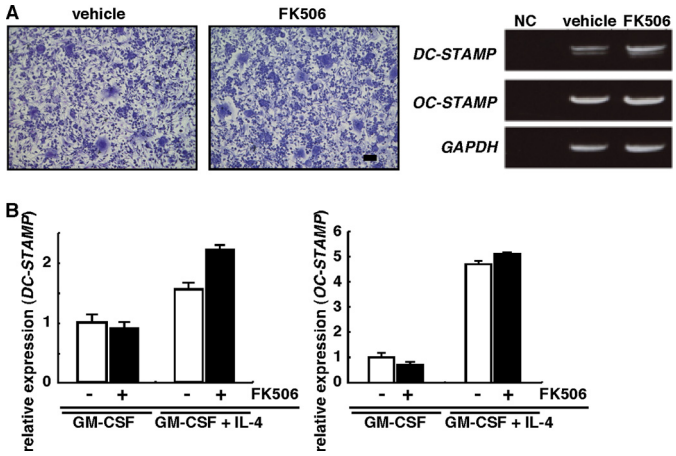

FIGURE 3. **OC-STAMP and *DC-STAMP* expression in FBGCs is NFAT-independent.** *A,* BMMs isolated from wild-type mice were cultured in the presence of GM-CSF (50 ng/ml) + IL-4 (50 ng/ml) with (FK506) or without (vehicle) FK506 (1 μM) for 6 days and stained with May-Grünwald Giemsa (*left panels*). *DC-STAMP* and *OC-STAMP* expression was analyzed by RT-PCR (*right panel*). *Bar,* 100 μm. *GAPDH* served as internal control. *NC,* no template control. *B,* BMMs isolated from wild-type mice were cultured in the presence of GM-CSF (50 ng/ml) or GM-CSF (50 ng/ml) + IL-4 (50 ng/ml) with (FK506) or without (vehicle) FK506 (1 μM) for 6 days, and *DC-STAMP* and *OC-STAMP* expression was analyzed by real time PCR.

OC-STAMP expression are both significantly inhibited by FK506, an NFAT inhibitor (19). By contrast, in FBGCs, FK506 treatment did not inhibit MMP9 protein expression nor did it alter multinuclear cell formation or *DC-STAMP* and *OC-STAMP* expression (Fig. 3, *A* and *B*, and supplemental Fig. S2). Thus, *DC-STAMP*/*OC-STAMP* expression is differently regulated in osteoclasts and FBGCs.

STAT1 Negatively Regulates Cell-Cell Fusion and DC-STAMP/OC-STAMP Expression in FBGCs—Next, we analyzed the role of STAT1 in FBGC fusion, because STAT1 is reportedly activated in dendritic cells induced following stimulation with GM-CSF plus IL-4 (29). STAT1 phosphorylation levels in cells treated with GM-CSF plus IL-4 was similar to that seen in cells treated with GM-CSF alone and was higher than in cells treated with M-CSF alone (Fig. 4*A*). Because STAT1 was phos-

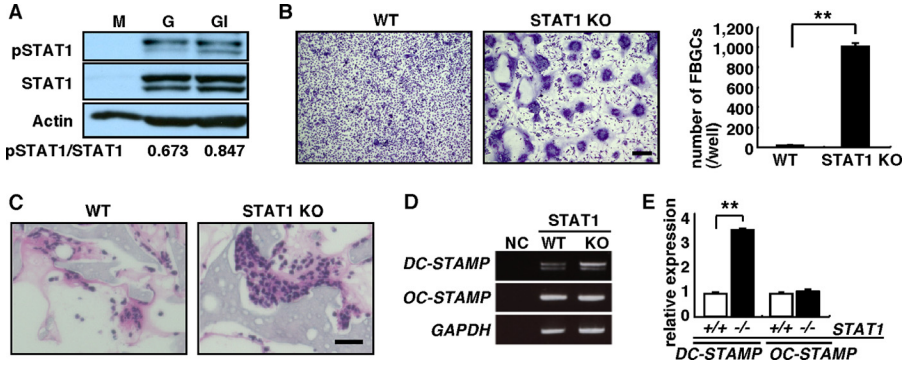

FIGURE 4. **STAT1 inhibits both cell-cell fusion and *DC-STAMP/OC-STAMP* expression in FBGCs.** *A,* BMMs isolated from wild-type mice were cultured in the presence of M-CSF (*M*), GM-CSF (*G*), or GM-CSF + IL-4 (*GI*) for 6 days, and STAT1 phosphorylation was analyzed by Western blot. The ratio of pSTAT1 to STAT1 signal intensity as analyzed by Western blot is shown as pSTAT1/STAT1. *B,* BMMs were isolated from wild-type (*WT*) and STAT1-deficient (*STAT1 KO*) mice and cultured in the presence of GM-CSF + IL-4 for 2 days. Cells were then stained with May-Grünwald Giemsa (*left panels*), and the number of multinuclear FBGCs was scored (*right panel*). **, $p < 0.001$. *Bar,* 50 μm. *C,* histological analysis of implants in STAT1-deficient and wild-type mice by hematoxylin and eosin staining. *Bar,* 50 μm. *D, DC-STAMP* and *OC-STAMP* expression in FBGCs derived from wild-type (*WT*) and STAT1-deficient (*KO*) mice was analyzed by RT-PCR. *GAPDH* served as an internal control. *NC,* no template control. *E, DC-STAMP* and *OC-STAMP* expression in FBGCs derived from wild-type (+/+) or STAT1-deficient (−/−) mice was analyzed by real time PCR. **, $p < 0.001$.

phorylated in wild-type FBGCs, we utilized STAT1-deficient mice to analyze the function of STAT1 in regulating FBGC formation. Interestingly, formation of multinuclear, MMP9-positive (supplemental Fig. S3) FBGCs was significantly up-regulated in STAT1-deficient mice *in vitro* and *in vivo* (Fig. 4, *B* and *C*). Although *OC-STAMP* expression was normal in STAT1-deficient cells, *DC-STAMP* expression significantly increased in STAT1-deficient FBGCs, indicating that STAT1 negatively regulates cell-cell fusion and *DC-STAMP* expression in FBGCs induced by GM-CSF plus IL-4 (Fig. 4, *D* and *E*).

STAT6-STAT1 Axis Regulates FBGC Cell-Cell Fusion via DC-STAMP/OC-STAMP—Multinuclear FBGCs were not formed, and only cell aggregates were formed in the presence of GM-CSF alone without IL-4 in wild-type cells (Fig. 5*A*). Interestingly, we observed formation of multinuclear FBGCs, which express MMP9 (supplemental Fig. S4), in the absence of IL-4 in STAT1-deficient cells (Fig. 5*A*). *DC-STAMP* and *OC-STAMP* expression, as analyzed by RT-PCR and real time PCR, significantly increased in STAT1-deficient cells compared with wild-type cells treated with GM-CSF without IL-4 (Fig. 5, *B* and *C*), suggesting that STAT1 inhibits both *DC-STAMP* and *OC-STAMP* expression in the presence of GM-CSF alone.

To understand mechanisms underlying IL-4-independent FBGC formation in STAT1-deficient cells, we examined signal transducer and activator of transcription factor protein phosphorylation. STAT1 deficiency did not enhance STAT6 phosphorylation in cells treated with GM-CSF alone, although in the presence of IL-4, STAT6 phosphorylation was equivalent in STAT1-deficient and WT cells (Fig. 5*D*). This observation indicates that STAT6 activation is strictly IL-4-dependent and that STAT1 does not regulate STAT6 phosphorylation in macrophages. By contrast, enhanced STAT1 phosphorylation was evident in STAT6-deficient FBGCs compared with wild-type FBGCs in the presence of GM-CSF plus IL-4 (Fig. 5*E*). We conclude that STAT6 is upstream of STAT1 in FBGCs and that it inhibits STAT1 activation.

DC-STAMP and OC-STAMP Co-expression Is Sufficient for Cell-Cell Fusion of Macrophages—Finally, we asked whether combined expression of OC-STAMP and DC-STAMP was sufficient for macrophage fusion in the absence of IL-4, because fusion correlates with increased *OC-STAMP* and *DC-STAMP* expression in STAT1-deficient macrophages in the presence of GM-CSF alone. BMMs isolated from wild-type or OC-STAMP-Tg mice were cultured in the presence of GM-CSF alone (Fig. 6*A*). In OC-STAMP Tg cells, *OC-STAMP* expression was driven by the actin promoter, whereas endogenous *DC-STAMP* expression was induced by GM-CSF in both OC-STAMP Tg and wild-type cells (Fig. 6*A*). In these conditions,

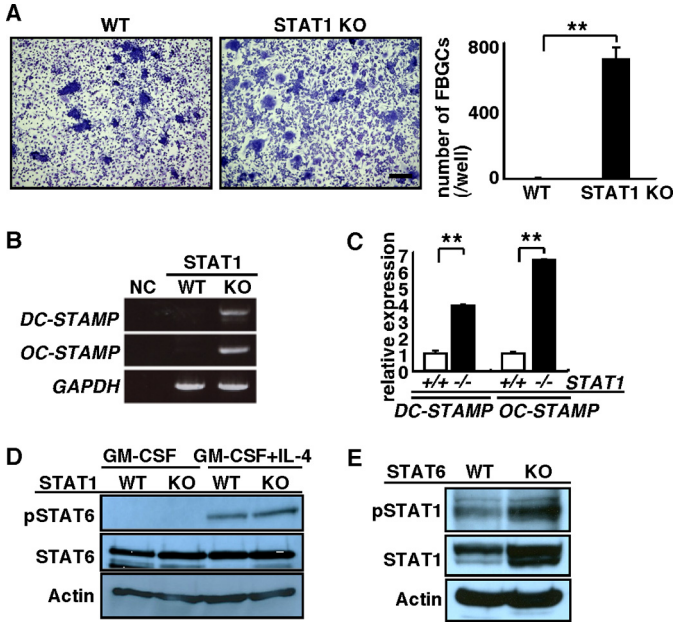

FIGURE 5. **STAT1 deficiency is sufficient to promote macrophage cell-cell fusion without IL-4.** *A–C,* BMMs were isolated from wild-type (*WT*) and STAT1-deficient (*STAT1 KO*) mice and cultured in the presence of GM-CSF alone for 7 days. Cells were then stained with May-Grünwald Giemsa (*A, left panels*), and the number of multinuclear FBGCs containing more than three nuclei was scored (*A, right panel*). **, $p < 0.001$. *Bar,* 50 μm, and *DC-STAMP* and *OC-STAMP* expression was analyzed by RT-PCR. *NC,* no template control (*B*) and by real time PCR. **, $p < 0.001$ (*C*). *D,* BMMs were isolated from wild-type (*WT*) and STAT1-deficient (*KO*) mice, cultured in the presence of GM-CSF alone or GM-CSF + IL-4 for 7 days, and analyzed for STAT6 phosphorylation by Western blot. *E,* BMMs were isolated from wild-type (*WT*) and STAT6-deficient (*KO*) mice, cultured in the presence of GM-CSF + IL-4 for 7 days, and analyzed for STAT1 phosphorylation by Western blot.

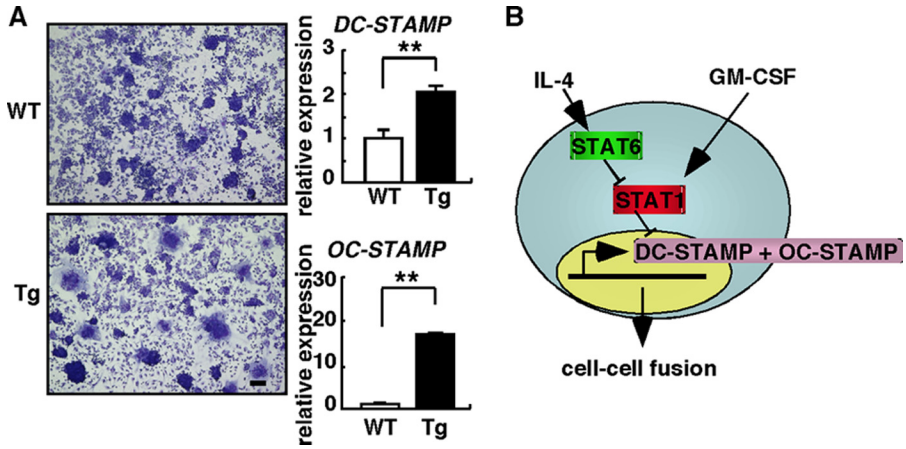

FIGURE 6. **DC-STAMP and OC-STAMP co-expression promotes macrophage fusion without IL-4.** *A,* BMMs were isolated from wild-type (*WT*) and OC-STAMP Tg (*Tg*) mice and cultured in the presence of GM-CSF alone for 7 days. Cells were stained with May-Grünwald Giemsa (*left panels*). *DC-STAMP* and *OC-STAMP* expression was analyzed by real time PCR (*right panels*). **, $p < 0.001$. *Bar,* 100 μm. *B,* schematic representation of induction of macrophage cell-cell fusion. GM-CSF induces STAT1 activation, although IL-4 inhibits it by activating STAT6. STAT1 inhibition induces *DC-STAMP* and *OC-STAMP* expression, which in turn promotes fusion of macrophages.

MMP9 expression was detected in both DC-STAMP Tg and wild-type cells (supplemental Fig. S5); however, cell-cell fusion occurred only in OC-STAMP Tg but not in wild-type cells (Fig. 6*A*). Although FBGC cell-cell fusion was completely abrogated in DC-STAMP-deficient and OC-STAMP-deficient cells (supplemental Fig. S6*A*), STAT6 phosphorylation was detected in DC-STAMP-deficient and OC-STAMP-deficient cells as wild-type FBGCs (supplemental Fig. S6*B*). Thus, OC-STAMP and DC-STAMP likely cooperate in promoting FBGC fusion through the STAT6-STAT1 axis (Fig. 6*B*).

DISCUSSION

Multinuclear giant cell formation occurs at sites of implantation or granulomatous inflammation and is a consequence of cell-cell fusion of mononuclear cells (1, 18, 22, 30). However, the mechanisms underlying such fusion have remained largely unknown. We previously identified two transmembrane proteins, OC-STAMP and DC-STAMP, which are essential for osteoclast and macrophage cell-cell fusion, and we showed that lack of either completely abrogated cell-cell fusion of both osteoclasts and FBGCs (18, 19). Here, we show that following IL-4 stimulation, the STAT6-STAT1 axis regulates *OC-STAMP* and *DC-STAMP* expression in FBGCs. STAT6 deficiency completely inhibited cell-cell fusion in FBGCs *in vivo* and *in vitro*. Lack of STAT6 increased STAT1 activation, which in turn significantly inhibited both *OC-STAMP* and *DC-STAMP* expression in FBGCs. Furthermore, STAT1 deficiency was sufficient to promote cell-cell fusion and *OC-STAMP* and *DC-STAMP* expression in FBGCs without IL-4, indicating that IL-4 signaling through STAT6 is likely required to inhibit STAT1 and promote fusion by inducing OC-STAMP and DC-STAMP. Indeed, OC-STAMP and DC-STAMP co-expression was sufficient to promote cell-cell fusion in macrophages not treated with IL-4. We conclude that *OC-STAMP* and *DC-STAMP* expression and the STAT6-STAT1 signaling cascade are essential for FBGC cell-cell fusion.

STAT1 and STAT6 reportedly reciprocally regulate each other in T cells (31). However, we found that in macrophages, STAT6 regulates STAT1 activation, whereas STAT6 activation is STAT1-independent, suggesting that regulation of signal transducer and activator of transcription factor protein activity differs depending on cell type.

Osteoclasts and FBGCs share common progenitors, and both express the factors such as MMP9, DC-STAMP, OC-STAMP, and ATP6v0d2 (13, 15, 18, 19). In addition, cell-cell fusion in both cells requires DC-STAMP and OC-STAMP (18, 19). However, their respective differentiation is induced by unique cytokine combinations as follows: M-CSF + RANKL for osteoclasts and GM-CSF + IL-4 for FBGCs. Both GM-CSF and IL-4 strongly inhibit osteoclastogenesis, suggesting that different signals govern fusion of osteoclasts and FBGCs. Osteoclast differentiation has been extensively characterized, and c-Fos, a component of AP1, and NFATc1 have been shown to be essential for osteoclastogenesis (32). Stimulation by RANKL, a cytokine essential for osteoclast differentiation, induces and activates NFATc1. We have shown that NFATc1 inhibition by FK506, a calcineurin inhibitor, inhibits cell-cell fusion as well as *DC-STAMP* and *OC-STAMP* expression in osteoclasts but not in FBGCs (Fig. 3) (19), suggesting that mechanisms underlying cell-cell fusion mediated by DC-STAMP and OC-STAMP differ in osteoclasts and FBGCs. Indeed, lack of STAT6 completely blocked fusion of FBGCs but not of osteoclasts. Overall, these observations suggest that DC-STAMP and OC-STAMP are required for fusion of osteoclasts and FBGCs via unique signal transducer and activator of transcription factor signaling.

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