**TREM-2 (triggering receptor expressed on myeloid cells 2) is a phagocytic receptor for bacteria**

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**Phagocytosis** is essential for the immune response to pathogens, initiated by specific interactions between pathogens and cell surface receptors expressed by phagocytes. This study identifies triggering receptor expressed on myeloid cells 2 (TREM-2) and its signaling counterpart DAP12 as a molecular complex that promotes phagocytosis of bacteria. Expression of TREM-2–DAP12 enables nonphagocytic Chinese hamster ovary cells to internalize bacteria. This function depends on actin cytoskeleton dynamics and the activity of the small guanosine triphosphatases Rac and Cdc42. Internalization also requires src kinase activity and tyrosine phosphorylation. In bone marrow–derived macrophages, phagocytosis is decreased in the absence of DAP12 and can be restored by expression of TREM-2–DAP12. Depletion of TREM-2 inhibits both binding and uptake of bacteria. Finally, TREM-2–dependent phagocytosis is impaired in Syk-deficient macrophages. This study highlights a novel role for TREM-2–DAP12 in the immune response to bacterial pathogens.

**Introduction**

Phagocytosis is an essential element of the innate immune response to infectious agents. Professional phagocytes engulf invading microorganisms, subjecting them to intracellular processes that typically lead to their degradation (Brown, 1995; Aderem and Underhill, 1999; Jutras and Desjardins, 2005). Internalized pathogens can be further exposed to pattern recognition receptors (e.g., Toll-like receptors), thereby activating additional pathways of the innate immune response (Underhill et al., 1999). Phagocytosis also contributes to antigen processing for presentation to T cells (Jutras and Desjardins, 2005).

Phagocytosis is initiated upon interaction between microorganisms and receptors expressed at the surface of phagocytes. These interactions are highly specific, and phagocytes express an elaborate arsenal of receptors that enables recognition of a wide variety of microorganisms. Once receptors are engaged, membrane remodeling at the site of interaction leads to the complete wrapping of the particle and its subsequent release in the cytoplasm within a membrane-bound compartment, the phagosome (Aderem and Underhill, 1999; Jutras and Desjardins, 2005). Membrane dynamics during phagocytosis are driven by a controlled rearrangement of the actin cytoskeleton (Groves et al., 2008).

Triggering receptors expressed on myeloid cells (TREM) are type I membrane proteins with an extracellular Ig-like domain and a short cytoplasmic tail that has no intrinsic signaling capacity (Klesney-Tait et al., 2006). TREM signaling relies on association with DAP12, a cytosolic adapter that also associates with other receptors. DAP12 contains an immunoreceptor tyrosine-based activation motif (ITAM), which becomes phosphorylated upon activation of DAP12–associated receptors. The phosphorylated ITAM in turn recruits and activates Syk tyrosine kinase, leading to cellular responses such as regulation of cytokine production (Lanier and Bakker, 2000; Takaki et al., 2006).

Previous work has shown that TREM-2 promotes phagocytosis of apoptotic neurons by microglia (Takahashi et al., 2005), although the underlying mechanisms remain unclear. Our prior work has shown that TREM-2 promotes phagocytosis of bacteria (Takahashi et al., 2005).

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work showed that TREM-2 binds a wide variety of bacteria (Daws et al., 2003). However, receptor ligation alone does not trigger phagocytosis by default. For example, the complement receptor 3 binds C3bi-coated particles but does not promote their internalization in nonactivated cells (Wright and Silverstein, 1982, 1983). Therefore, we examined whether TREM-2 binding to bacteria promotes their phagocytosis. This study demonstrates that TREM-2–DAP12 but not TREM-1–DAP12 functions as a phagocytic receptor for bacteria.

**Results and discussion**

**Expression of TREM-2 with DAP12 promotes phagocytosis of bacteria by CHO cells**

To examine the role of TREMs in phagocytosis, we individually expressed TREM-1 and TREM-2 in a nonphagocytic cell line, CHO. This approach was used by others to characterize the binding and/or phagocytosis-promoting functions of receptors, including Fc (Downey et al., 1999) and complement (Cywes et al., 1996) receptors. We anticipated that TREM function would require DAP12, the only adapter known to mediate TREM signaling (Takaki et al., 2006; Turnbull and Colonna, 2007). To ensure this, however, we expressed a chimeric molecule in which the TREM extracellular domain was covalently linked to DAP12 cytoplasmic tail (Fig. 1 A). These constructs recapitulate the functions of the endogenous TREM–DAP12 complexes (Hamerman et al., 2006). TREM–DAP12 cDNAs were expressed from a GFP bicistronic vector, allowing the use of GFP fluorescence as an indicator of their expression (Fig. 1 B).

CHO cells expressing TREM-1–DAP12 (T1D12), TREM-2–DAP12 (T2D12), or DAP12 only (D12) were challenged with *Escherichia coli* coupled to Alexa Fluor 594 (594–*E. coli*), and the interaction was monitored by microscopy. T2D12 cells showed substantial association with 594–*E. coli*, whereas T1D12 or D12 cells did not (Fig. 1 C, top). A live *E. coli* strain internally expressing the fluorescent protein AsRed also showed increased association with T2D12 cells but not with T1D12 or D12 cells, ruling out an interaction caused by the Alexa Fluor dye (Fig. 1 C).

To determine whether the bacteria associated with T2D12 cells were extracellular or internalized, cells challenged with 594–*E. coli* were fixed using a nonpermeant fixative and stained with *E. coli* antibodies (Fig. 1 D). Both single- and double-stained bacteria colocalized with T2D12 cells, indicating that TREM-2–DAP12 promoted both binding and uptake of *E. coli*. Pretreatment of T2D12 cells with cytochalasin D (cyto D), which inhibits actin polymerization and blocks phagocytosis (Graves et al., 2008), abolished colocalization of single-stained bacteria with T2D12 cells, whereas double-stained bacteria bound to cells could still be detected (Fig. 1 D). Electron micrographs showed internalized bacteria found in phagosomes (Fig. 1 E, right) and cell-bound bacteria surrounded by pseudopods (Fig. 1 E, left). Thus, TREM-2–DAP12 mediates both binding and actin-dependent uptake of *E. coli* by CHO cells.

To strengthen and quantify these results, phagocytosis was also analyzed by flow cytometry, measuring the fluorescence of 594–*E. coli* associated with T2D12 cells. Consistent with the microscopy results, expression of TREM-2–DAP12 promoted *E. coli* association with CHO cells (Fig. 1 F). Cyto D–treated T2D12 cells showed stronger association with *E. coli* than did T1D12 or D12 cells, confirming that *E. coli* still bound T2D12 cells in the absence of phagocytosis.

To examine the generality of TREM-2–DAP12 function, association of T2D12 cells with a variety of particles was examined. *Francisella tularensis* (a Gram negative) and *Staphylococcus aureus* (a Gram positive) were each internalized through TREM-2 (Fig. 1 G), as was *Pseudomonas aeruginosa* (another Gram-negative pathogen; not depicted), suggesting the existence of a broad range of phagocytic substrates for TREM-2. In contrast, zymosan particles (derived from the cell wall of *Saccharomyces cerevisiae*, a nonpathogenic yeast) did not interact with T2D12 cells (Fig. 1 G), indicating a degree of ligand specificity. Previously, TREM-2 binding to the yeast *Candida albicans* was found to be minimal (Daws et al., 2003). Because the cell wall composition of *C. albicans* is very similar to that of *S. cerevisiae* (Firon et al., 2004), it is not unexpected that TREM-2 would not bind zymosan. In contrast to T2D12 cells, T1D12 cells did not support binding or uptake of any of the particles tested.

**TREM-2-dependent phagocytosis in CHO cells requires tyrosine phosphorylation and src kinase activity and is enhanced by Syk**

To initiate a signaling cascade, TREM receptors depend on their association with the ITAM-containing adapter DAP12 (Klesney-Tait et al., 2006). Upon TREM binding, phosphorylation of the ITAM by src kinases allows the recruitment of Syk tyrosine kinase (Lanier and Bakker, 2000; Takaki et al., 2006). We examined the role of these signaling components. Pretreatment of T2D12 cells with genistein (tyrosine kinase inhibitor) or PP2 (src family kinase inhibitor) reduced bacterial uptake, whereas inhibition of myosin light chain kinase did not (Fig. 2 A). Furthermore, a T2D12 construct in which a mutation in the ITAM inhibited its phosphorylation did not interact with T2D12 cells (Fig. 1 G), indicating a degree of ligand specificity. Previously, TREM-2–DAP12 signaling through Syk ITAM has been described previously (Takaki et al., 2006). However, our results indicate that significant TREM-2–dependent phagocytosis occurs in the absence of exogenous Syk (Fig. 1), which raises the possibility that Syk might be endogenously expressed in CHO cells or that a related kinase might be involved. Similar observations were made in CHO cells expressing the Fc receptor FcγRIIA: substantial phagocytosis was observed in the absence of exogenous Syk (Downey et al., 1999). However, other studies in leukocytes revealed an essential role for Syk in FcγRIIA-dependent uptake (Crowley et al., 1997; Kiefer et al., 1998). Syk is primarily expressed in myeloid cells.
Figure 1. Expression of TREM-2–DAP12 promotes binding and internalization of bacteria by nonphagocytic cells. (A) Schematic of TREM association with DAP12 in trans [endogenous conformation, left] or through covalent linkage [chimeric construct, right]. (B) CHO cells were transfected with bicistronic constructs containing GFP together with TREM-1–DAP12 (T1D12) or TREM-2–DAP12 (T2D12) or DAP12 only (D12). Surface expression of TREM–DAP12 chimeras in GFP-positive cells was verified by flow cytometry using antibodies to TREM-1 or TREM-2. (C) T1D12, T2D12, or D12 cells were challenged with 594–E. coli (top) or AsRed–E. coli (bottom). Association of transfected cells with bacteria was analyzed by fluorescence microscopy and quantified (graph). (D) T2D12 cells challenged with 594–E. coli were stained with E. coli antibodies and analyzed by microscopy. Pseudocolors: blue, T2D12 cells; red, 594–E. coli; green, anti-E. coli staining. Internal bacteria are red, whereas extracellular bacteria are both red and green (yellow on merge). Both internalized and extracellular cell-bound bacteria were scored (graph). Arrowheads represent cell-bound bacteria. (E) T2D12 cells challenged with live, unlabeled E. coli were analyzed by electron microscopy. Both binding (left) and internalization of bacteria within phagosomes (right) were observed. Arrowheads, bacteria; asterisks, pseudopods. (F) Association of T2D12 cells with 594–E. coli was analyzed by flow cytometry and quantified by measuring the fluorescence intensity of bacteria associated with GFP-positive cells. Representative plots are shown. Internalization of bacteria (but not binding) was blocked with cyto D. Fluorescence intensities were normalized to values of parental cells. The graph represents the mean of four experiments ± SEM. *, P < 0.05; **, P < 0.01 relative to parental values. (G) T1D12, T2D12, or D12 cells were challenged with F. tularensis, S. aureus, or zymosan, and their association was quantified as in F. Fluorescence intensities were normalized to D12 values in each set. The graph represents the mean of three experiments ± SEM. *, P < 0.05; **, P < 0.01 relative to D12 values. Bars: (C and D) 10 μm; (E) 1 μm.
TREM-2 – DAP12-dependent phagocytosis in CHO cells requires Rac1 and Cdc42 GTPases of the Rho family play essential roles in phagocytosis: rearrangement of actin-rich formations in the vicinity of the particle leads to its internalization by the surrounding membrane (Niedergang and Chavrier, 2005). Pretreatment of T2D12 cells with \textit{Clostridium difficile} toxin B, an inhibitor of the Rho family of GTPases, reduced uptake of \textit{E. coli} (Fig. 3 A). Consistent (Berton et al., 2005) but is also found in nonmyeloid tissues (Yanagi et al., 2001). Indeed, phagocytosis by T2D12 cells was inhibited by the Syk inhibitor piceatannol (Fig. 2 D), but because this inhibitor may act on other kinases, any conclusion about the identity of the kinase involved in CHO cells requires further investigation. Our results nonetheless indicate that Syk positively regulates TREM-2 function in phagocytosis by CHO cells.
with this, phagocytosis was abrogated by inhibition of PI-3 kinase (Fig. 3 A), an activator of GTPases (Andrews et al., 2007) required for pseudopod extension during phagocytosis (Cox et al., 1999), but not by inhibition of DNA–protein kinase (Fig. 3 A). During phagocytosis, pseudopod formation and membrane expansion around the particle relies on Rac and Cdc42 (Caron and Hall, 1998), whereas formation of stress fibers beneath the bound particle and subsequent membrane retraction depend on Rho activity (Olazabal et al., 2002). The contribution of each GTPase to phagocytosis through TREM-2 was assessed through expression of dominant-negative (dn) constructs (Fig. 3 B). dn Rac1 and dn Cdc42 inhibited E. coli uptake, whereas dn RhoA had a marginal effect (Fig. 3 B). This shows a major role for Rac1 and Cdc42 and is consistent with electron micrographs showing pseudopod formation around bacteria (Fig. 1 D; Caron and Hall, 1998).

**TREM-2 contributes to phagocytosis of bacteria by macrophages**

Results obtained in CHO cells identified TREM-2–DAP12 as a molecular complex that promotes phagocytosis of bacteria. To validate this observation in professional phagocytes, we first focused on DAP12-deficient (DAP12−/−) macrophages. Association of both E. coli and F. tularensis with DAP12−/− bone marrow–derived macrophages (BMDMs) was reduced compared with wild-type (wt) BMDMs, whereas that of zymosan was
DAP12-associated molecules in addition to TREM-2 (but excluding TREM-1) might also participate in phagocytosis. Finally, TREM-2 levels on BMDMs were reduced by the introduction of a short hairpin RNA (Fig. 5A; Hamerman et al., 2006). TREM-2 knockdown (KD) cells showed both reduced binding and uptake of bacteria (Fig. 5B), which is consistent with a role for TREM-2 in promoting both functions (Fig. 1; Daws et al., 2003). Quantification revealed a 25% reduction in internalization (Fig. 5C). Binding and phagocytosis were also examined in TREM-2−/−/H11002 BMDMs, which allows a direct assessment of TREM-2 relative contribution to phagocytosis. As with TREM-2 KD, both binding and uptake of E. coli were decreased in the absence of TREM-2 (Fig. 5D). Under these comparable (Fig. 4A). In contrast, no difference was found in binding (Fig. 4B), indicating that the reduced association of bacteria with DAP12−/− cells reflects a decreased uptake but not a reduced binding. A possible increased bacterial degradation in DAP12−/− BMDMs was ruled out by the use of bafilomycin A1, an inhibitor of lysosomal fusion. Furthermore, we verified that TREM-2 was still expressed on DAP12−/− BMDMs (Fig. 4C). Importantly, reintroduction of either DAP12 alone or TREM-2−DAP12 into DAP12−/− BMDMs restored phagocytosis, whereas TREM-1−DAP12 did not (Fig. 4D). Thus, the decreased uptake by DAP12−/− BMDMs can be attributed, at least in part, to an impaired TREM-2 signaling. However, because DAP12 is a signaling adapter for various receptors (Takaki et al., 2006), other DAP12-associated molecules in addition to TREM-2 (but excluding TREM-1) might also participate in phagocytosis.

Finally, TREM-2 levels on BMDMs were reduced by the introduction of a short hairpin RNA (Fig. 5A; Hamerman et al., 2006). TREM-2 knockdown (KD) cells showed both reduced binding and uptake of bacteria (Fig. 5B), which is consistent with a role for TREM-2 in promoting both functions (Fig. 1; Daws et al., 2003). Quantification revealed a 25% reduction in internalization (Fig. 5C). Binding and phagocytosis were also examined in TREM-2−/− BMDMs, which allows a direct assessment of TREM-2 relative contribution to phagocytosis. As with TREM-2 KD, both binding and uptake of E. coli were decreased in the absence of TREM-2 (Fig. 5D). Under these
Figure 5. **TREM-2 promotes phagocytosis of bacteria by macrophages in a Syk-dependent manner.** [A] Cellular levels of TREM-2 on BMDMs were decreased by the expression of a short hairpin RNA (TREM-2 KD) and measured by flow cytometry. An isotype antibody was used as a negative control. MFI, mean fluorescence intensity. [B] *E. coli* association with control or TREM-2 KD BMDMs at 4 or 37°C was assessed by flow cytometry. [C] Bacteria uptake was quantified by subtracting 4°C from 37°C fluorescence values. The graph represents the mean of five experiments ± SEM. ***, P ≤ 0.001. [D and E] *E. coli* association with wt or TREM-2−/− BMDMs was assessed as in B, and bacteria uptake was quantified in E as described in C. The graph represents the mean of three experiments ± SEM. **, P ≤ 0.01. [F] TREM-2-D12 or DAP12 alone (mutated in its transmembrane domain) was expressed in wt or Syk−/− BMDMs, and phagocytosis of *E. coli* or zymosan was quantified by subtracting 4°C from 37°C fluorescence values. The graph represents the mean of three experiments ± SEM. *, P ≤ 0.05 between wt/mutated DAP12 and wt/TREM-2-DAP12. AU, arbitrary unit.
conditions, ~30% of phagocytosis could be attributed to TREM-2 (Fig 5 E). Altogether, these data demonstrate a role for TREM-2 in the nonopsonic uptake of E. coli by macrophages.

Results in CHO cells suggested the involvement of Syk or of a related kinase in phagocytosis through TREM-2 (Fig 2). In BMDMs, overexpression of TREM-2–DAP12, which increased E. coli uptake by wt cells, had no effect on phagocytosis of E. coli by Syk−/− cells (Fig 5 F). In contrast, deletion of Syk did not inhibit zymosan uptake as previously reported (Herre et al., 2004; Underhill et al., 2005). This indicates that Syk is required for TREM-2 function in phagocytosis by BMDMs.

We have identified a novel phagocytosis pathway initiated by ligation of TREM-2, which involves DAP12 phosphorylation and Syk activation. The expression of TREM-2 was previously shown to promote uptake of apoptotic neurons by microglia (Takahashi et al., 2005), although the underlying mechanisms and associated signaling events are unknown. Recent work by Ziegenfuss et al. (2008) shows that Draper, a Drosophila melanogaster phagocytic receptor, promotes uptake of apoptotic bodies by microglia through activation of its intrinsic ITAM and recruitment of the Syk orthologue, Shark. Thus, the association of phagocytosis with this signaling cascade has been preserved throughout evolution.

TREM-2–DAP12 has also been shown to regulate the production of reactive oxygen species in response to Salmonella typhimurium by macrophages, although TREM-2 binding to S. typhimurium could not be demonstrated (Charles et al., 2008). Thus, TREM-2 may perform additional innate immune functions without binding to pathogens, possibly by engaging endogenous ligands. This study is the first demonstration that the TREM-2–DAP12 complex is fully competent to promote internalization of bacteria by both nonphagocytic cells and professional phagocytes. TREM-2 is also a negative regulator of Toll-like receptor signaling initiated by microbial components (Hamerman et al., 2006). Therefore, TREM-2 function in both phagocytosis and Toll-like receptor pathways might allow a fine-tuning of the macrophage response to infection.

**Materials and methods**

**Cell culture and transfection**

CHO cells were maintained in RPMI 1640 + 10% FCS, transfected using Lipofectamine 2000 (Invitrogen), and used 24 h after transfection. BMDMs were derived from femurs of sex- and age-matched wt or knockout mice (C57BL/6 background) as described previously (Roach et al., 1997). DAP12-deficient mice (provided by L. Lanier, University of California, San Francisco, CA) and TREM-2-deficient mice were previously described (Bokker et al., 2000; Turnbull et al., 2006). Syk-deficient cells were obtained as described previously (Mocsai et al., 2006). Cells were maintained in DME with 10% FCS and 10% CMG14-12 cell culture supernatant with parental cells or was restricted to transduced BMDMs or associated bacteria was determined. Analysis was performed on the whole population with parental cells or was restricted to transduced BMDMs or transfected CHO cells (expressing GFP in each case) as indicated. A quantitative estimation of phagocytosis was obtained by subtracting extracellular fluorescence from total bacterial fluorescence.

**Phagocytosis assays**

Cells were transfected into HBSS + 0.1% BSA for 1 h. 1 μM bafilomycin A1 was added to prevent lysosomal degradation of internalized particles. To block phagocytosis and to assess bacteria binding, 2 μM cyto D was added in control wells for 10 min. Alternatively, cells were transfected to 4°C to allow particle binding but not internalization. Particles at an MOI of 10:1 (BMDM) or 50:1 (CHO) were spun onto cells at 500 g for 3 min, and phagocytosis assays were performed for 60 min in a 37°C incubation chamber. For analysis by microscopy, cells challenged with 594–E. coli were fixed with 3.7% PFA and stained with an Alexa Fluor 647 secondary antibodies. Particles at an MOI of 10:1 (BMDM) or 50:1 (CHO) were spun onto cells at 500 g for 3 min, and phagocytosis assays were performed for 60 min in a 37°C incubation chamber. For analysis by microscopy, cells challenged with 594–E. coli were fixed with 3.7% PFA and stained with an E. coli antibody (Invitrogen). The number of extracellular cell-bound (double stained) and intracellular (single stained) bacteria was scored on 50 cells. For analysis by flow cytometry (FACSCalibur; BD), the mean fluorescence intensity of cell-associated bacteria was determined. Analysis was performed on the whole population with parental cells or was restricted to transduced BMDMs or transfected CHO cells (expressing GFP in each case) as indicated. A quantitative estimation of phagocytosis was obtained by subtracting extracellular fluorescence from total bacterial fluorescence.

**Microscopy imaging**

Bacteria colocalization with cells (Fig 1 C) was analyzed with a fluorescence microscope (Eclipse TE300; Nikon) with a 60x objective. Images were acquired with a camera (CoolSNAP HQ2; Photometrics) using Simple PCI software (Compix). Triple-staining experiments (Fig 1 D) were analyzed with a fluorescence confocal microscope (LSM510, Carl Zeiss, Inc.) using a 63x objective.

**Retrovirus-mediated transduction in BMDMs**

cDNAs (D12, T1D12, and T2D12) were introduced into BMDMs by using retroviral infection as described previously (Hamerman et al., 2006). The packaging cell line Plat-E (provided by T. Kitamura, University of Tokyo, Tokyo, Japan; Morita et al., 2000) was transfected with retroviral constructs using Lipofectamine 2000. Virus-containing supernatants were collected 48 h later, added onto 3-d marrow cells in the presence of 4 μg/ml polybrene, and incubated for at least 3 d before assays. Retrovirus-mediated depletion of endogenous TREM-2 was obtained as previously described (Hamerman et al., 2006).

**Reagents**

TREM antibodies were purchased from R&D Systems. Syk antibody was purchased from Cell Signaling Technology. Cyto D and bafilomycin A1 were obtained from Sigma-Aldrich. Genistein, PP2, C. difficile toxin, LY29004, ML-7, and DNA–protein kinase inhibitor were purchased from EMD. Alexa Fluor particles and dye and E. coli antibodies were obtained from Invitrogen.

**DNA constructs**

TREM–DAP12 and DAP12 cDNAs were previously described (Hamerman et al., 2006), and Flag-Syk cDNA (provided by I. Frasier, California Institute of Technology, Pasadena, CA) was previously described (Zavazadjan et al., 2007). Myc-tagged dn RhoA, Rac1, and Cdc42 were generated in the laboratory of G. Bokoch (The Scripps Research Institute, La Jolla, CA) and distributed by Addgene.

**Bacteria**

Alexa Fluor 594 or 488 particles (zymosan, E. coli, and S. aureus) were purchased from Invitrogen. AlexaFluor–GFP–E. coli were provided by the Sandia National Laboratories. E. vulneris strain U112 was provided by D. Monack (Stanford University, Stanford, CA). All cultures were grown to midlog phase. Alexa Fluor 594 staining of F. tularensis was performed according to the manufacturer’s instructions. Bacterial viability after staining was assessed by dilution plating and found to be comparable with that of unstained bacteria.

**Phagocytosis assays**

Cells were transfected into HBSS + 0.1% BSA for 1 h. 1 μM bafilomycin A1 was added to prevent lysosomal degradation of internalized particles. To block phagocytosis and to assess bacteria binding, 2 μM cyto D was added in control wells for 10 min. Alternatively, cells were transfected to 4°C to allow particle binding but not internalization. Particles at an MOI of 10:1 (BMDM) or 50:1 (CHO) were spun onto cells at 500 g for 3 min, and phagocytosis assays were performed for 60 min in a 37°C incubation chamber. For analysis by microscopy, cells challenged with 594–E. coli were fixed with 3.7% PFA and stained with an E. coli antibody (Invitrogen). The number of extracellular cell-bound (double stained) and intracellular (single stained) bacteria was scored on 50 cells. For analysis by flow cytometry (FACSCalibur; BD), the mean fluorescence intensity of cell-associated bacteria was determined. Analysis was performed on the whole population with parental cells or was restricted to transduced BMDMs or transfected CHO cells (expressing GFP in each case) as indicated. A quantitative estimation of phagocytosis was obtained by subtracting extracellular fluorescence from total bacterial fluorescence.

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**TREM surface staining**

CHOs were fixed with PFA and incubated with 5 μg/ml TREM-1 or TREM-2 antibodies for 30 min at 4°C followed by 2 μg/ml Alexa Fluor 647 secondary antibodies for 30 min at 4°C. TREM surface staining was analyzed by flow cytometry.

**Syk and myc intracellular staining**

CHO cells transfected with Flag-Syk or myc-tagged RhoA, Rac1, and Cdc42 were detached, fixed in PFA, permeabilized with −20°C methanol, incubated with 5 μg/ml anti-Syk or 2.5 μg/ml anti-myc for 30 min at 4°C, and then incubated with 2 μg/ml Alexa Fluor 647 secondary antibodies. Intracellular staining was measured by flow cytometry.

**Electron microscopy**

T2D12 cells incubated with E. coli for 60 min were fixed in Karnovsky’s fixative, postfixed in reduced OsO4, and stained with uranyl-acetate. After ethanol dehydration and clearing in propylene oxide, cells were embedded in eponate 12 (Ted Pella Co.). Thin sections were examined under a transmission electron microscope (Tecnai 10; Philips).

**Statistical analysis**

All samples were analyzed in duplicates. Experiments were repeated at least three times, and statistical significance was determined by using the Student’s t test.
References


