CD47, a Ligand for the Macrophage Fusion Receptor, Participates in Macrophage Multinucleation*

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The macrophage fusion receptor (MFR), also called P84/BIT/SIRPα/SHP-1, is a transmembrane glycoprotein that belongs to the superfamily of immunoglobulins. Previously, we showed that MFR expression is highly induced at the onset of fusion in macrophages, and that MFR appears to play a role in macrophage-macrophage adhesion/fusion leading to multinucleation. The recent finding that IAP/CD47 acts as a ligand for MFR led us to hypothesize that it interacts with CD47 at the onset of cell-cell fusion. CD47 is a transmembrane glycoprotein, which, like MFR, belongs to the superfamily of immunoglobulins. We show that macrophages express the hemopoietic form of CD47, the expression of which is induced at the onset of fusion, but to a lower level than MFR. A glutathione S-transferase CD47 fusion protein engineered to contain the extracellular domain of CD47, binds macrophages, associates with MFR, and prevents multinucleation. CD47 and MFR associate via their amino-terminal immunoglobulin variable domain. Of the nine monoclonal antibodies raised against the extracellular domain of CD47, three block fusion, as well as MFR-CD47 interaction, whereas the others have no effect. Together, these data suggest that CD47 is involved in macrophage multinucleation by virtue of interacting with MFR during adhesion/fusion.

Osteoclasts and giant cells are characterized by multinucleation and a powerful ability to resorb the substrate onto which they adhere. Although osteoclasts and giant cells play an important role in bone remodeling and immune defense, respectively, they are also associated with osteoporosis, granulomatous diseases, and tumors.

Multinucleation appears to endow macrophages with the capacity to digest and resorb extracellular infectious agents, foreign material, and other components that are too large to be internalized, such as bone. This resorption occurs in an "extracellular lysosomal compartment" sealed off between the multinucleated cell and its target substrate (reviewed in Ref. 1). The plasma membrane that faces that extracellular domain is highly ruffled and specialized. Multinucleation gives macrophages added resorptive capacity, in part by making available a large excess of plasma membrane.

Understanding the mechanism by which macrophages differentiate into osteoclasts and multinucleated giant cells is of extreme importance. One of the key steps in the differentiation of osteoclasts and giant cells is the fusion mechanism of their mononucleated precursor cells. It is assumed that both osteoclasts and giant cells originate from the fusion of mononuclear phagocytes. Despite the pathophysiological importance of these cells, the mechanism by which their mononucleated precursors fuse remains poorly understood. Indeed, cell-cell fusion itself, whether it concerns that of sperm with oocytes in fertilization or myoblasts with myoblasts in muscle development, has not been investigated thoroughly. It is proposed that cell-cell fusion involves a set of proteins similar to those used by viruses to fuse with host cells before injecting their DNA or RNA (2). It has been hypothesized that viruses have usurped the fusion protein machinery from their target cells. It is now well accepted that virus-cell fusion requires both an attachment mechanism and a fusogenic peptide. One such example is human immunodeficiency virus attachment, where gp120 binds CD4 on T lymphocytes and macrophages (3, 4), whereas the fusion molecule gp40 triggers the actual fusion event. Although putative fusion molecules mediating sperm-oocyte and myoblast fusion have been reported (5–8), the actual protein machinery governing the attachment and fusion of these cells remains unknown.

To investigate the mechanism of homotypic mononuclear phagocyte fusion leading to the differentiation of osteoclasts and giant cells, our hypothesis has been that macrophage-macrophage fusion, similar to virus-cell fusion, depends on the expression of specific cell surface proteins. To identify such proteins, we had established an in vitro macrophage fusion assay as a model system. When cultured under fusogenic conditions, rat alveolar macrophages rapidly generate large polykaryons whose non-adherent plasma membrane is enriched in sodium pumps while the opposite plasma membrane facing the substrate is enriched in proton pumps (9, 10). This is a property that is shared with osteoclasts (reviewed in Ref. 1). Using these fusing macrophages as immunogen, we previously generated four monoclonal antibodies (mAbs)1 that block fusion.

1 The abbreviations used are: mAb, monoclonal antibody; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; MHC, major histocompatibility complex; PCR, polymerase chain reaction; nt, nucleotide(s); GST, glutathione S-transferase; ELISA, enzyme-linked
sition. All four mAbs recognize the same antigen, the macrophage fusion receptor (MFR), which is highly and transiently induced at the onset of fusion (11, 12). MFR was cloned simultaneously by several groups as P84/BIT/SIRPo1/SHPS-1 (13–15). MFR is a type I transmembrane glycoprotein that belongs to the superfAMILY OF IMMUNOGLOBULINS (Ig) (12). MFR contains three Ig domains in its extracellular part and closely resembles CD47. The intracellular domain of MFR associates with the phosphatases SHP-1 and SHP-2, hence its name, SHPS-1 (15). We reported that the recombinant extracellular domain of MFR engineered as a GST fusion protein blocks fusion by specifically binding to fusogenic macrophages (12), suggesting that MFR interacts with a putative ligand expressed on the surface of fusing macrophages. Jiang et al. (16) recently reported that IAP/CD47 is a ligand for P84/BIT known to promote neurite outgrowth (17), suggesting that CD47 might be the relevant MFR ligand in macrophage fusion. Seiffert et al. (18) demonstrated that CD47 is a counterreceptor for human SIRPo1. CD47 is a widely expressed 50-kDa protein that belongs to the superfAMILY OF IMMUNOGLOBULINS and was initially identified through co-purification with the integrin αb3 from human placenta (19) prior to being shown to be CD47 (20, 21). CD47 comprises an extracellular immunoglobulin variable domain and five transmembrane domains with its COOH-terminal domain located intracellularly. Its intracellular COOH-terminal domain exists in four alternatively spliced forms (22).

We reasoned that if macrophages express CD47, this molecule might interact with MFR and participate in macrophage adhesion/fusion leading to multinucleation. We present evidence that: (i) fusing macrophages express the hemopoietic form of CD47, (ii) a fusion protein engineered to contain the extracellular domain of CD47 blocks fusion, (iii) three out of nine mAbs directed against the extracellular domain of CD47 block both MFR-CD47 interaction and fusion, and (iv) CD47 and MFR interact via their immunoglobulin variable domain in fusing macrophages. Together, these data suggest that CD47 belongs to the protein machinery that mediates macrophage adhesion/fusion leading to multinucleation.

**EXPERIMENTAL PROCEDURES**

**Cells—** Rat alveolar macrophages were obtained from 12-week-old Fisher 344 rats (Charles River, Kingston, NY) by tracheobronchial lavage and cultured in fusogenic conditions as described previously (9, 10). In brief, cells were plated at a density of 5 × 10⁶ cells/ml in MEME supplemented with 10% human serum, and then, once adherent, cultured in 5% human serum.

**Antibodies—** Mouse anti-CD47 monoclonal antibodies were generated by immunizing IAP-deficient mice with purified human placental CD47 (19), fusing spleen cells with the non-secreting myeloma P3 × 63Ag8.653 (ATCC), and screening clones for reactivity with human and monkey cells. The antibodies were the miap, mouse monoclonal antibody anti-IAP/CD47. Immunoassay using IAP-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml). Following four washes of 15 min each in PBS-milk, the cells were incubated for 2 h at room temperature in PBS-milk supplemented with mouse anti-CD47 mAbs (2 μg/ml), mAb 10C4 (cell culture supernatant, 1:100), or control mouse IgG2a (2 μg/ml).
Moss Inc., Pasadena, MD). Optical density (OD_{650}) measurements were made using a kinetic reader (Menlo Park, CA).

Immunoprecipitation, Pull-down, and SDS-PAGE Analysis—Rat alveolar macrophages were harvested by lavage, plated in six-well plates at 5 × 10^6 cells/ml, cultured in MEME supplemented with 5% HS for 2 days, and immunoprecipitated as described above. Both pull-downs and immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions following Western blot analysis using mAb 10C4 and miap 460-biotin, then HRP-rat anti-mouse F(ab')2 and mAb 10C4 and miap 450. In contrast, the signal detected on most of the blots exposed to x-ray films.

Production of GST-MFRev: Recombinant Soluble Extracellular V1 Domain of MFR (MFRev) Fused to GST—GST-MFRev, as expressed as a fusion protein system (Amersham Pharmacia Biotech). PCR amplification of the extracellular domain of MFR was performed using the primers that contained the NcoI and BamHI sites. The resulting fragment was cloned into the pGEX-4T-1 vector (Amersham Pharmacia Biotech). The recombinant plasmid was transformed into E. coli strain BL-21. Soluble GST-MFRev was isolated from 2 liters of bacterial culture using the bulk GST purification module as described by the manufacturer’s instructions. The eluted protein was extensively dialyzed against PBS. Recombinant GST-MFRev ran as a fusion protein of approximately 39 kDa. Its concentration was determined by running a 5-μl aliquot on a 10% SDS-polyacrylamide gel and staining with Coomassie Brilliant Blue. For protein concentration determination, the intensity of the stained band was tested against that determined by running a 5-μl aliquot on a 10% SDS-polyacrylamide gel and staining with Coomassie Brilliant Blue. For protein concentration determination, the intensity of the stained band was tested against that determined by running a 5-μl aliquot on a 10% SDS-polyacrylamide gel and staining with Coomassie Brilliant Blue. For protein concentration determination, the intensity of the stained band was tested against that determined by running a 5-μl aliquot on a 10% SDS-polyacrylamide gel and staining with Coomassie Brilliant Blue.
To determine whether the cell surface expression of CD47, like MFR, was altered by multinucleation, fusing macrophages were subjected to ELISA at different time points after plating. As shown previously, the expression of MFR is highly induced at the onset of multinucleation, i.e. 24 h after plating, and decreases thereafter with multinucleation (12). CD47 expression was also induced 1 day after plating, but to a lesser extent than MFR (Fig. 1B), and continued to increase through day 2. Thereafter, like MFR, CD47 expression tended to decrease with multinucleation. These results were confirmed by Western blot analysis of fusing macrophage lysates at various times after plating (Fig. 1C). When CD47 was analyzed under non-reducing conditions, it ran as an 87–91-kDa doublet, probably due its multimerization. Of importance, mAb 10C4 (anti-MFR) recognized an additional band of 90 kDa that was most obvious 24 h after plating, indicating that it was also induced. Aliquots of the same cell lysates were analyzed in parallel by Western blot under reducing conditions using a polyclonal antibody that

lesser amount of CD47. This is a pattern of expression similar to that of MFR (11).
recognizes the intracellular domain of MFR. The 90-kDa protein was again detected, at low abundance, but was induced with kinetics similar to that of the 150-kDa MFR (Fig. 1C, lower panel). Although the 90-kDa protein could represent a degradation product of MFR, it may also correspond to the short transcript that lacks exons 3 and 4 encoding the second and third immunoglobulin domains (25, 26). To confirm the even loading of proteins in each lane, the blot was reprobed with an antibody directed against the tyrosine phosphatase SHP-1 whose abundance remained unaffected by multinucleation (Fig. 1C, lower panel). Together, our results show that CD47 expression is transiently induced in fusing macrophages, but to a lower level than both forms of MFR, e.g. the 150-kDa and 90-kDa forms.

Because CD47 is expressed as four different isoforms that differ from each other at their cytoplasmic carboxyl termini (22), we sought to determine which CD47 isoform was expressed by fusing macrophages. Total RNA from fusing macrophages was subjected to reverse transcriptase-PCR using primer pairs designed from the rat CD47 cDNA sequence to generate a full-length CD47 cDNA. The PCR product was then subjected to DNA sequencing. This analysis revealed that the dominant form of CD47 expressed by fusing macrophages is isofrom 2, which also predominates in bone marrow cells (data not shown).

Monoclonal Antibodies Anti-CD47 Block Fusion/Multinucleation—The identification of CD47 as a MFR ligand suggested that it might play a role in fusion. To investigate this possibility, we tested whether our anti-CD47 mAbs inhibited macrophage fusion. Among the nine mAbs tested, three blocked fusion in a concentration-dependent manner (miaps 430, 450, and 470) (Fig. 2, A and B; Table I; and data not shown). mAb miap 400 is representative of the 6 miaps that did not alter multinucleation (Fig. 2A, and data not shown). Indeed, this is not unexpected since other anti-human CD47 mAbs can be divided into two groups based on whether or not they inhibit function: in this case, in the regulation of Fc receptor-dependent phagocytosis (19). This suggested that different mAbs miaps recognize distinct sites on the CD47 Ig domain.

Recombinant GST-CD47e Fusion Protein Blocks Fusion/Multinucleation—To further investigate the role of CD47 in macrophage fusion, we engineered a GST fusion protein that contains the NH2-terminal extracellular Ig variable domain of CD47 (GST-CD47e) and tested its ability to bind fusing macrophages. As shown in Fig. 3 (A and B), GST-CD47e bound fusing macrophages in a concentration-dependent, saturable, and reversible manner. When GST-CD47e was added to fusing macrophages, multinucleation was inhibited in a concentration-dependent manner with a maximal effect at 20 nM (Fig. 3C), a potency similar to that of GST-MFRev (12). In contrast, GST did not block fusion. This suggested that the domain of MFR important for macrophage fusion/multinucleation interacts with CD47. Indeed, the inhibition of fusion by anti-CD47 mAbs suggested that CD47 is actively involved in cell-cell interaction. These results are consistent with a role for CD47-MFR interaction in macrophage adhesion/fusion leading to multinucleation.

MFR and CD47 Associate in Fusing Macrophages—To investigate whether CD47 associates with MFR, we took two complementary approaches. First, we subjected lysates from fusing macrophages to immunoprecipitation using anti-CD47 mAb and tested for co-precipitation with MFR by subjecting the precipitate to SDS-PAGE in non-denaturing conditions followed by Western blotting using anti-MFR mAb 10C4. The blot was reprobed with biotinylated anti-CD47 mAb miap 460 to confirm the precipitation of CD47. As a control, MFR was immunoprecipitated with a rabbit polyclonal antiserum that

![Image](image_url)
MFRev (containing the V loop of MFR, see below, and Fig. 4)
coupled to glutathione-Sepharose beads. The lysates were first pre-cleared using GST alone coupled to glutathione-Sepharose beads. The material pulled down was analyzed by SDS-PAGE in non-denaturing conditions followed by Western blot as above, using mAb 10C4 and miap 460-biotin, respectively. Together, these experiments revealed that CD47 and MFR associate with each other (Fig. 4, A and B), indicating that MFR interacts with CD47 from fusing rat alveolar macrophages. Of significance, only anti-CD47 mAbs that did not block fusion co-precipitated MFR with CD47 (data not shown). This suggested that mAbs that block fusion do so by preventing CD47-MFR interaction, and that MFR interacts with CD47 during cell-cell adhesion/fusion. Although the mAb anti-CD47 miap 460 precipitated both forms of MFR, GST-MFRev pulled down the 150-kDa form only. This could suggest that the IgV domain of MFR interacts with a domain of CD47 that is not represented in GST-CD47e, or else simply reflect differences in yield since the 90-kDa form of MFR is rather less abundant than the 150-kDa form, hence not detected.

The Immunoglobulin V1 Domain of MFR Is Sufficient to Block Multinucleation—The fact that the 90-kDa form of MFR was recognized by both mAb 10C4 and a polyclonal antibody directed against the intracellular domain of MFR strongly sug-

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**Fig. 3.** GST-CD47e binds fusing alveolar macrophages and blocks fusion. Alveolar macrophages were plated at 5 x 10⁶/ml in 96-well plates, cultured in MEME supplemented with 5% human serum for 24 h, fixed with 4% paraformaldehyde, blocked in PBS plus 5% milk, and incubated overnight with GST-CD47e at the indicated concentrations for saturation binding studies (a) and at 20 nM for dissociation binding studies (b). Binding of the fusion proteins was revealed using HRP-conjugated mouse anti-GST. c, cells were cultured as in a, and the medium was supplemented with GST, GST-MFRev, and GST-CD47e at the indicated concentrations. Original magnification, x100.

**Fig. 4.** MFR and CD47 associate. a, alveolar macrophages were plated at 5 x 10⁶/ml in six-well plates, 1.2 x 10⁶ cells/well, cultured in MEME supplemented with 5% human serum for 24 h. The cells were lysed and subjected to immunoprecipitation (IP) using anti-CD47 miap 460 and anti-MFR. The lysates were analyzed by SDS-PAGE in non-reducing conditions followed by Western blot using mAb 10C4 (MFR) and biotin-miap 460 (CD47). b, cells were plated and cultured as in a, but the lysates were subjected to pull-down procedure using GST-CD47e or GST-MFRev coupled to glutathione-Sepharose 4B beads, after pre-clear with GST alone coupled to glutathione-Sepharose 4B beads. The pull-downs were analyzed by SDS-PAGE in non-reducing conditions followed by Western blot using mAb 10C4 (MFR) and biotin-miap 460 (CD47). Total cell lysates (TCL) were analyzed in parallel. c, GST-MFRev and GST recombinant proteins (1 µg/lane) were analyzed by SDS-PAGE in non-reducing conditions followed by Western blot using mAb 10C4 (MFR) and goat anti-GST.
suggested that the short form of MFR contained only one extracellular Ig linked to the intracellular domain (12, 25–27). The fact that both forms of MFR co-immunoprecipitated with CD47 suggested that it is the single IgV domain present in the short form of MFR that interacts with CD47. To test this possibility, we engineered a GST fusion protein that contained the IgV domain of MFR fused to GST (GST-MFRev). As shown in Fig. 3C, GST-MFRev blocked multinucleation with a potency similar to that of GST-MFRe, i.e., 20 nM (12). mAb 10C4 (anti-MFR) also blocks fusion, and as expected immunoblots GST-MFRev (Fig. 4C), showing that it recognizes the amino-terminal IgV domain of MFR. Together, these data show that the IgV domain of MFR plays an important role in macrophage multinucleation by interacting with CD47.

**MFR and CD47 Interact in Fusing Macrophages**—To investigate whether CD47 interacts with MFR in situ, in intact fusing cells, we performed competitive binding studies between the mAbs and the GST fusion proteins that block fusion in macrophages cultured under fusogenic conditions for 24 h, then fixed. Cells were incubated for 1 h at 4°C with increasing concentrations of either mAb 10C4 or miap 450, both of which block fusion. Either GST-CD47e or GST-MFRev were then added and detected using HRP-anti-GST antibody. As shown in Fig. 5A, both mAb 10C4 and miap 430 prevented the binding of the recombinant proteins GST-CD47e and GST-MFRev, respectively, in a concentration-dependent manner. This indicated that the antibodies and the ligands bind to the same epitopes, and suggested that they inhibit fusion by preventing MFR-CD47 interaction. Of importance, miaps 430 and 470, which block fusion, gave similar results, whereas miaps that did not block fusion failed to prevent GST-MFRe binding to macrophages (Fig. 5B, and data not shown). Together, these results suggest that MFR and CD47 interact via their variable domain in fusing macrophages.

We then reasoned that if MFR and CD47 interact during fusion, the kinetics of GST-MFRe and GST-CD47e binding sites expression on fusing macrophages should mirror that of their ligand, i.e., CD47 and MFR previously detected by ELISA and Western blot analysis (Fig. 1, B and C). To verify this possibility, macrophages were cultured in fusogenic conditions for increasing amounts of time and subjected to ELISA using GST-MFRe and GST-CD47e as ligands, as described in Fig. 3 (A and B). GST fusion proteins were detected as described above. The data presented in Fig. 5C confirm that the kinetics of GST-MFRe and GST-CD47e binding sites expression is similar to that of CD47 and MFR expression.

**DISCUSSION**

Our hypothesis has been that macrophage fusion, like virus-cell fusion, is mediated by a set of surface proteins that interact in a ligand-receptor manner. The identification of the ligand for MFR was a key step to further our understanding of the fusion mechanism in macrophages. We have now generated evidence that CD47 plays a role in macrophage adhesion/fusion leading to multinucleation by virtue of interacting with MFR. CD47, like MFR, belongs to the superfamily of immunoglobulins. We report here that CD47 and MFR interact with each other, at least in part, via their immunoglobulin variable domain to promote macrophage multinucleation.

An important consideration regarding macrophage fusion is that it involves a homotypic interaction, in contrast to the interaction occurring between neurons at synaptic sites, and between viruses with host cells. This implies that plasma membranes of both cells are endowed with the same set of molecules and interact in a reciprocal manner (Fig. 6). With this reasoning, it is attractive to speculate that the short form of MFR, although poorly expressed, brings the opposite plasma mem-

![Fig. 5. Competitive binding between MFR and CD47.](image-url)
plasma membranes down to 5–10 nm. Although neither MFR nor CD47 show any homology with known viral fusion proteins, they could potentially facilitate fusion by utilizing one system that combines two functions, attachment and fusion. Accordingly, MFR and CD47 may constitute the "minimal fusion machinery" proposed by Weber et al. (28) for intracellular membrane fusion. Meanwhile, the trans-association between the short form of MFR and CD47 may secure the mononucleated status of macrophages.

Fig. 6. Model for macrophage adhesion/fusion. Macrophage-macrophage adhesion is secured by MFR and CD44, interacting together directly or indirectly, or with other unknown ligands (X and Y). The stepwise association between the long form of MFR with CD47 is followed by the short form of MFR with CD47, which could reduce the gap between the cells down to 5 to 10 nm. That distance may be further reduced if MFR and CD47 bend upon binding. We propose a macrophage adhesion/fusion model in which MFR and CD47 interact with each other via their IgV domain, as co-receptors, suggesting that MFR and CD47 entertain a receptor-ligand type of interaction. This model allows for possible additional ligands for MFR and CD44. We propose that cell-cell adhesion/fusion utilizes one system that combines two functions, attachment and fusion. Accordingly, MFR and CD47 may constitute the "minimal fusion machinery" proposed by Weber et al. (28) for intracellular membrane fusion. Meanwhile, the trans-association between the short form of MFR and CD47 may secure the mononucleated status of macrophages.

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Addendum—Since this manuscript was submitted for publication, two articles that report on the interaction between MFR and CD47 have been published (35, 36).
CD47 and Macrophage Multinucleation

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