Complement Receptor of the Ig Superfamily Enhances Complement-Mediated Phagocytosis in a Subpopulation of Tissue Resident Macrophages

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Complement Receptor of the Ig Superfamily Enhances Complement-Mediated Phagocytosis in a Subpopulation of Tissue Resident Macrophages

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An important function of the complement cascade is to coat self and foreign particles with C3-proteins that serve as ligands for phagocytic receptors. Although tissue resident macrophages play an important role in complement-mediated clearance, the receptors coordinating this process have not been well characterized. In the present study, we identified a subpopulation of resident peritoneal macrophages characterized by high expression of complement receptor of the Ig superfamily (CRIg), a recently discovered complement C3 receptor. Macrophages expressing CRIg showed significantly increased binding and subsequent internalization of complement-opsonized particles compared with CRIg negative macrophages. CRIg internalized monovalent ligands and was able to bind complement-opsonized targets in the absence of Ca2+ and Mg2+, which differs from the β2-integrin CR3 that requires divalent cations and polyvalent ligands for activation of the receptor. Although CRIg dominated in immediate binding of complement-coated particles, CRIg and CR3 contributed independently to subsequent particle phagocytosis. CRIg thus identifies a subset of tissue resident macrophages capable of increased phagocytosis of complement C3-coated particles, a function critical for immune clearance. The Journal of Immunology, 2008, 181: 7902–7908.

Phagocytosis, an important component of host defense and a primary function of macrophages, is facilitated by opsonization, a process by which serum components tag pathogens and immune complexes for recognition by neutrophils and macrophages (1). Triggering of the classical, lectin, and alternative pathways of complement activates the central component C3 resulting in covalent binding of C3b to a particle with the release of C3a. C3b is rapidly degraded to iC3b by the action of factor I and cofactors and further degraded to C3c, C3d, and C3dg (2, 3). Binding of the C3dg and C3d fragments to CR2 on B cells leads to enhanced Ab production (4), whereas binding of C3 proteins to complement receptors (CRs); on macrophages results in phagocytosis and clearance of the particle (5–8).

CR3, a transmembrane heterodimer composed of two integrin subunits (CD11b or αM and CD18 or β2), is a well established CR for the dominant C3 cleavage product, iC3b, and in mice is present on all myeloid cells and a subset of NK cells (7). CR3-mediated phagocytosis is dependent on receptor redistribution and conformational changes, two processes that require activating stimuli (9, 10). Upon activation of phagocytes with phorbol esters (11) or β glucan (12, 13), cell surface expression and activation state of CR3 are increased, enabling CR3-mediated phagocytosis. Similarly, inflammatory macrophages freshly recruited from the circulation to peritoneal cavity use CR3 for phagocytosis (14, 15). Although tissue resident macrophages are thought to play an important role in both initiation and resolution of inflammation (16–18), CR3 does not act as a potent phagocytic CR on these cells (19).

Recently, we identified a novel CR of the Ig superfamily (CRIg) that is highly expressed on Kupffer cells (20, 21). CRIg is required for rapid sequestration of blood-borne Listeria monocytogenes and Staphylococcus aureus in a C3-dependent manner, thereby limiting systemic bacteremia and promoting survival of the host. In this study, we show that CRIg is required for efficient complement-mediated phagocytosis by non-activated as well as activated resident peritoneal macrophages (RPMs). Thus, CRIg identifies a subset of tissue macrophages that is capable of integrin-independent rapid recognition and phagocytosis of iC3b-opsonized particles in the absence or presence of inflammatory stimuli, a feature of importance for tissue homeostasis and host defense.

Materials and Methods

Abs, proteins, and dyes

Non-blocking mAbs against CRIg were generated as described (20). Blocking Abs (clone 2H1 and 14G8; Genentech) were generated by immunizing CRIg-deficient mice with CRIg-Fc fusion protein generated as described (20). Phycoerythrin-labeled anti-F4/80 Ab (F4/80-PE) was purchased from Caltag Laboratories. Unless indicated, all other Abs were purchased from BD Biosciences. C4b was purchased from Complement Technologies. Human C3, C3b, and iC3b (used in Fig. 2) were obtained from fresh serum and purified as described (20). Murine C3 was purified from fresh mouse serum using the same method. Murine C3b was generated by cleavage of purified C3 by trypsin as described elsewhere (22). Murine iC3b was purified from pooled murine ascites obtained from the
i.p. cavity following implantation of various hybridomas. Ascites was buffered with 25 mM Tris (pH 7.5), 5 mM EDTA, and 100 mM NaCl. PEG 6000 was added to the ascites to a final concentration of 10% and continuously mixed for 1 h. This solution was centrifuged at 20,000 × g for 15 min. The supernatant was decanted, and the pellet was re-suspended in 50 mM Tris (pH 8.6) and 5 mM EDTA and loaded over a protein G column to deplete the IgG from the solution. The flow through was applied directly onto a mono Q (10 × 100; GE Healthcare), which was eluted with a gradient of 0 to 0.7 M NaCl over 20-column volumes. The murine iC3b was isolated and loaded separately over a Superdex 200 (16/60; GE Healthcare) at 7 cm/h in 25 mM Tris (pH 8.0), 150 mM NaCl, and 5 mM EDTA. The resulting fractions were analyzed by SDS-PAGE, and identity was confirmed by Edman degradation.

Abs and proteins were labeled with Alexa Fluor 555 (A555), Alexa Fluor 488 (A488), or Alexa Fluor 647 (A647) according to manufacturer’s instruction (Invitrogen). CR1g-ECV was generated by expression of the extracellular domain of murine CR1g in Chinese hamster ovary cells and purified as described (20). All other reagents are from Sigma-Aldrich unless noted.

**Animals and flow cytometry analysis of resident peritoneal macrophages**

All animals were held under sterile pathogen free conditions and animal experiments were approved by the Institutional Animal Care and Use Committee of Genentech. Six to 7-wk old C57BL/6d mice and AKR mice were purchased from Jackson ImmunoResearch Laboratories. C3-deficient mice have been described (23). Resident peritoneal cells were lavaged with PBS containing 2 mM EDTA, resuspended in PBS containing 1% BSA, and incubated on ice with anti-mouse CD16/32 and purified rat IgG2a and IgG2b to block Fc receptors followed by A647-conjugated anti-CRIg Ab (clone 17C9), PE-conjugated Ab to F4/80, anti-CR1/CR2 (clone 7G6), anti-CR3 (CD11b, clone M1/70), anti-CD18, anti-CR4 (CD11c, clone HL3), or anti-CR-related gene Y (Crry; clone 1F2). After incubation with 7-amino-actinomycin D (7-AAD) (Molecular Probes), cells were analyzed by flow cytometry on a FACSCalibur (BD Biosciences). For immunohistochemistry of CR1g, CR1g F4/80+ and CR1g F4/80− nonadherent RPMs (naRPMs) were sorted (FACSria 1; BD Biosciences) and cultured in Lab-Tek II chamber slides (Nunc) in DMEM (High glucose DMEM (HGDMEM); Cellgro) containing 10% heat inactivated FBS (HyClone), 4.5 mg/ml glucose, 10 mM HEPES, 10 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) (HGDMEM-10). Cells were fixed with acetone/ethanol solution (75/25, v/v) at −20°C for 10 min, blocked for FcR-mediated binding as indicated above, and stained for CR1g as described (20).

**Binding and internalization of complement proteins by adherent RPMs (adRPMs)**

For microscopic evaluation of iC3b, C3b, and C3 internalization, cells were incubated in Lab-Tec II chamber slides (Nunc) for 1 h at 37°C in HGDMEM, nonadherent cells were removed and, after overnight, culture cells were stained with an A555-labeled anti-CRIg Ab (clone 14G8), incubated with A488-labeled complement proteins, and internalization visualized by microscopy.

**Binding and phagocytosis of sheep RBC (SRBC)**

SRBCs (5 × 10^7/ml; Colorado Serum) were labeled with rat IgM-A488 anti-Forsman Ab (clone TIB-123, 5 μg/ml; ATCC) for 30 min at 22°C (IgM-coated erythrocyte; E-IgM). 2.5 × 10^6 E-IgM were incubated with 1 ml Veronal Buffer (BioWhittaker) containing 10% serum from C5-deficient mice (The Jackson Laboratory, 0.15 mM CaCl_2, 0.1 mM MgCl_2, and 0.1% gelatin for 30 min at 37°C to generate complement-opsomized E-IgM (E-EC). Serum from AKR/J mice was used to prevent lysis of the SRBCs. For analysis of C3 fragments present on the EACs, EACs were lysed in 1% NP40 at 1E8/ml, an equal volume of 2X gel loading buffer plus 10% β-mercaptoethanol was added to yield a final cell lysis concentration of 5ET/ml. SES EACs in a volume of 10 μl were loaded per well. 25 μL A488-anti-α/βCR1g (clone 3F7) and rabbit anti-goat Ab (Thermo Fisher Scientific).

Binding experiments were performed on freshly lavaged, naRPMs resuspended in PBS/0.5% BSA. Cells were placed on ice and FcRs were blocked as described above plus 25 μg/ml anti-CRIg (clone 14G8), anti-CR1b (clone M1/70), or isotype control (anti-ragweed; Genentech) blocking Abs. EACs were added to the naRPMs in a 20:1 ratio for 30 min at 37°C and the amount of EACs bound determined by flow cytometry as described below. To determine the effect of divalent cations on binding,

![FIGURE 1. CR1g and CR3 are the only phagocytic complement C3 receptors expressed on RPMs.](http://www.jimmunol.org/)

naRPMs were resuspended in PBS/0.5% BSA with either 10 mM EDTA or 0.15 mM Ca^{2+}/1 mM Mg^{2+} and incubated with EACs for 30 min at 37°C. To determine the binding and phagocytosis of EACs to adRPMs, 1 × 10^6 peritoneal cells were placed in 6-well tissue culture plates (Costar) or in chamber slides in HGDMEM-10, nonadherent cells were removed after 1 h incubation at 37°C, and adherent cells were cultured overnight. Where indicated, adRPMs were activated with 1 μg/ml LPS (Escherichia coli strain O26:B6; Sigma-Aldrich) for 24 h. Cells were placed on ice, washed twice with cold PBS, and FcRs were blocked with cold HGDMEM/5 μg/ml anti-CD16/32 (BD Biosciences), 20 μg/ml anti-ragweed mlgG2a and mlgG2b (Genentech) for 10 min on ice. FcR blocking solution was then aspirated and 50 μg/ml anti-CRIg (14G8), anti-CD11b (clone M1/70), or isotype control Abs was added for 10 min on ice to block CRs. A total of 2 × 10^5 EACs in cold HGDMEM was added and binding synchronized by spinning at 500 rpm for 1 min at 4°C. Cells were placed at 37°C for indicated times and phagocytosis was stopped by placing cells on ice. To monitor phagocytosis by flow cytometry, cells were washed in PBS/10 mM EDTA, scraped off, transferred to chilled FACS tubes, washed 1X with PBS/0.5% BSA, and stained with F4/80-PE and anti-CRIg A647 (clone 25C5) for 25 min. Extracellular SRBCs were lysed with 1 ml of ACK lysis buffer (82 mM NH_4Cl, 5 mM KHCO_3, and 50 mM EDTA) for 2 min on ice, washed 2X with PBS, and then incubated with 30 μg/ml anti-A488 Ab (Invitrogen) to quench fluorescence of A488-conjugated EACs bound to the adRPM cell surface. Cells were analyzed on FACSCalibur, 1 μg/ml propidium iodide was added to exclude dead cells, and data analyzed with FlowJo software (TreeStar).
For immunofluorescence, adRPMs with EACs were washed with PBS/0.15 mM CaCl₂/1 mM MgCl₂, FcRs were blocked for 10 min and the blocking solution was then removed, and, finally, cells were stained with 3/26g/ml A555-conjugated CRig (clone 14G6) Abs for 15 min on ice. In some experiments, nonfluorescent EACs and 3/26g/ml anti-CD11b-FITC was used. Cells were washed and extracellular EACs lysed with ACK buffer for 2 min. In experiments where A488-conjugated EACs were used, the non-internalized EAC membrane was quenched with an anti-A488 Ab. adRPMs were fixed with 2% paraformaldehyde (EM Sciences) in PBS for 20 min and cover-slipped with VectaShield with 4/26,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories).

Fluorescence and deconvolution microscopy

Images were acquired on a Nikon Eclipse TE300 inverted microscope using a ×60 objective, a Zeiss LSM 510 confocal microscope using a 60× objective, or an Olympus BX61 upright microscope using a ×60 objective.

**Statistical analysis**

Statistical analysis was performed using JMP software (JMP Release: 5.1.2; SAS Institute). All p values are calculated with an unpaired Student’s t test assuming unequal variance.

**Results**

**CRig is expressed on a subpopulation of RPMs**

Both CRig and CR3 (or αMβ2) are receptors for iC3b present on serum-opsonized pathogens. To determine the relative contribution of CRig and CR3 to complement-mediated phagocytosis, we focused our studies on RPMs that express high levels of CR3. CRig was coexpressed with CR3 on 30% of the total population of RPMs (Fig. 1A) in line with a previous report (24). Next to expression on the cell surface, CRig was also localized on an intracellular pool of recycling endosomes, as described in our previous studies (25).

**Fluorescence and deconvolution microscopy**

Sequential images were acquired with a spacing of 0.2 microns along the z-axis and then deconvolved using softWoRx software (Applied Precision).
and CR3 in the phagosomes surrounding the EACs. Scale bar indicates nuclear staining with DAPI. Arrows indicate accumulation of CRIg.

CR1g and CR3 differ in their requirement for binding to complement-opsonized particles

To distinguish the contribution of CR1g and CR3 to binding and phagocytosis of particles coated with multivalent C3 proteins, IgM-opsonized SRBC were incubated with C5-deficient serum, obtained from AKR/J mice, to generate complement C3-coated SRBC (EACs). Flow cytometry analysis indicated deposition of C3 proteins on the surface of EACs that were incubated with C5-deficient serum but not on EACs incubated with C5-deficient serum (Fig. 3A, left panel). The major C3 protein coated on the surface of these particles was in the form of iC3b as illustrated by Western blot analysis using purified C3 proteins as a reference (Fig. 3A, right panel). Binding experiments were conducted using naRPMs. Under the conditions used and in line with a previous report (29), all A488-conjugated EACs bound to the surface of the naRPMs were not internalized as shown by the reduction of fluorescence to baseline values following lysis of these EACs and naRPMs. Under the conditions used and in line with a previous report (26), all A488-conjugated EACs bound to the surface of the naRPMs were not internalized as shown by the reduction of fluorescence to baseline values following lysis of these EACs and naRPMs.

Figure 4. CRIg and CR3 localize to phagosomes. adRPMs cultured overnight were stained with an anti-CR3 (green) or anti-CRIg (red) Ab before (A), or following (B), incubation with EACs for 30 min. Blue indicates nuclear staining with DAPI. Arrows indicate accumulation of CRIg and CR3 in the phagosomes surrounding the EACs. Scale bar = 20 µm.

Study (20). CRIg+ and CRIg− macrophages did not differ in shape or granularity as indicated by similar forward- and side-scatter profiles (Fig. 1B). CR3 and Cry, the latter acting as a regulator of complement activation, were uniformly expressed on CRIg+ and CRIg− RPMs (Fig. 1C) (25). Neither CRIg+ nor CRIg− RPMs expressed CR1, CR2, or CD11c, the α-chain of CR4 in accordance with a previous report (26). To establish whether CRIg+ and CRIg− RPMs represent different maturation stages of peripheral blood-derived monocytes entering the peritoneal cavity from the periphery, RPMs were FACS-sorted, cultured separately for 1 wk, permeabilized, and stained for CRIg. CRIg+ RPMs maintained CRIg expression on the cell surface and in the cytoplasm for at least 7 days in culture, whereas CRIg− RPMs neither expressed cell surface nor cytoplasmic CRIg (Fig. 1D). Thus, CRIg is stably expressed on a subpopulation of tissue resident macrophages that also expresses CR3.

CRIg is required for binding and internalization of monomeric C3-derived proteins

Since iC3b is the predominant C3 fragment found on complement-opsonized particles (27), we determined the contribution of CRIg vs CR3 in binding and internalization of soluble iC3b. For binding studies, freshly lavaged, naRPM were used. When incubated at 4°C, iC3b bound only to CRIg+ naRPMs (Fig. 2A). C3 did not bind to either CRIg+ or CR3+ macrophages in line with CRIg’s selective recognition of a neoepitope exposed on cleaved, but not native, C3 (21). CRIg blocking Ab or a soluble CRIg protein significantly reduced iC3b binding to RPMs while CR3 blocking Ab or CRIg isotype control Ab had no effect on binding activity (Fig. 2B), confirming the requirement of CRIg, and not CR3, for binding to monomeric iC3b. For studying internalization of C3 proteins, freshly lavaged RPMs were cultured overnight to generate adRPMs. When incubated with cultured adRPMs at 37°C, the bound ligands C3b and iC3b were internalized and colocalized with CRIg+ vesicles in the areas surrounding the nucleus as well as at the tips of macrophage pseudopodia (inset, Fig. 2C). Residual uptake of C3 in CRIg+ adRPMs was observed and likely indicates that some of the C3 was hydrolyzed and subsequently internalized through CRIg. CRIg+ adRPMs, identified by nuclear staining only (Fig. 2C, arrows), did not internalize any of the C3 proteins. These results illustrate that CRIg is able to bind and internalize monomeric ligands while CR3 is not, confirming the requirement of a multivalent ligand for CR3-mediated binding and internalization of target particles (9, 10, 28).

CRIg and CR3 differ in their requirement for binding to complement-opsonized particles

To further elucidate the subcellular localization and contribution of CRIg and CR3 to particle phagocytosis, adRPMs, capable of phagocytosing complement-opsonized particles (27), were used.
CR3 was distributed equally on all adRPMs while CR1g, as expected, localized to a subset of adRPMs (Fig. 4A). To participate in all steps of the phagocytic process, the receptors involved have to redistribute to the site of particle contact (30). Upon incubation with EACs, CR1g and CR3 redistributed to localize in the phagosome surrounding the EAC (Fig. 4B). To further determine whether CR1g and CR3 colocalization with phagosomes translates into more efficient phagocytosis, adRPMs were incubated with A488-fluorescently labeled EACs (Fig. 5A) followed by dissociation of the phagocytes from the culture dish. Phagocytosis was determined by FACS analysis after lysis of cell surface-bound EACs. Remaining fluorescence resulting from lysed EAC bound to the cell surface was quenched with a polyclonal anti-A488 Ab. As a result, internalized EACs were the primary source of fluorescence. Uptake of EACs was significantly increased in CRIg+ adRPMs compared with CRIg− adRPMs (Fig. 5A). In CRIg− adRPMs incubated with EACs for 30 min, CR1g and CR3 each contributed to ~50% of total phagocytosis which was reduced to background levels when both receptors were simultaneously blocked (Fig. 5B, left panel). In CRIg− adRPMs, as expected, phagocytosis was entirely mediated through CR3 (Fig. 5B, right panel). These results indicate that CR1g and CR3 act independently and additively to enhance binding and phagocytosis of complement C3-opsonized particles. To determine whether CR1g blocking Ab disrupts trafficking of CR1g to the forming phagosome, cultured adRPMs incubated with EACs were treated with isotype or CR1g-blocking Abs and then stained with a nonblocking Ab to CR1g. In the presence of isotype control Ab, CR1g accumulated in the forming phagosome (Fig. 5C, upper panels). Addition of CR1g-blocking Ab prevented CR1g accumulation in the phagosome and resulted in even distribution of CR1g on the cell surface (Fig. 5C, lower panels). This indicates that blocking CR1g binding to its ligand iC3b prevents CR1g accumulation in the phagosome and inhibits phagocytosis of the complement-opsonized particles.

In vivo, tissue macrophages often encounter various activating stimuli, including bacterial cell wall products and cytokines (31). To determine whether CR1g could act as a phagocytic receptor once the macrophages were activated, adRPMs were incubated overnight with LPS. This stimulation resulted in increased expression of the activation marker CD86, while CR1g expression was slightly reduced (Fig. 6A). In the activated macrophages, particle internalization was evident only after 20 min (Fig. 6B). Similar to nonactivated macrophages, LPS-activated CR1g+ adRPMs showed significantly increased phagocytosis at 30 min, contributed to by both CR1g and CR3 (Fig. 6C). Thus, CR1g significantly contributes to increased complement-mediated phagocytosis in both nonactivated and LPS-activated adRPMs.

**Discussion**

CR1g was initially described as a Kupffer cell-expressed CR required for clearance of pathogens from the circulation (20). This study shows that, besides Kupffer cells, CR1g is expressed on a
CRIg is expressed and functional on LPS-activated adRPMs. A, adRPMs cultured overnight in the presence of 1 μg/ml LPS were incubated with A488-conjugated EACs as described in the legend of Fig. 5. FACS analysis was performed using an Ab to CD86, CD11b, and CRIg. B, Phagocytosis of A488-labeled EACs by LPS-activated adRPMs was determined at various time points as described in the legend of Fig. 5. C, LPS-activated adRPMs were incubated with A488-conjugated EACs for 30 min in the presence of isotype or CRIg and/or CR3 blocking Abs. The amount of phagocytosed EACs in CRIg+ and CRIg− adRPMs was then determined by staining the cells with a non-blocking Ab to CRIg and measuring the MFI of A488 using flow cytometry. Results are expressed as mean ± SD, n = 3, *p < 0.05; **p < 0.01.

subpopulation of RPMs. These mononuclear phagocytes are capable of local synthesis of complement components (2, 32), enabling opsonization of target particles in the absence of direct exposure to serum. In the current study, we demonstrate that the two CRs expressed on these macrophages, CRIg and CR3, independently contribute to complement-mediated phagocytosis of iC3b-opsonized particles. We further illustrate that the requirements for binding and ingestion of target particles differ between CRIg+ and CRIg− RPMs.

CRIg accelerates complement-mediated phagocytosis by rapidly binding and internalizing opsonized particles independent of receptor crosslinking, activating stimuli or divalent cations. Thus, CRIg functions differently from CR3/αMβ2 integrin, which firmly binds and internalizes opsonized targets only after exposure to additional stimuli such as phorbol esters, chemokines, and TNF-α that induce major conformational changes in both the α- and β-chains (10, 33). Although CRIg colocalizes with CR3 on a subpopulation of RPMs, CRIg is absent from inflammatory macrophages infiltrating the peritoneum following a challenge with the irritant thioglycollate (24) (results not shown). In the infiltrating cells, CR3 is the dominant phagocytic receptor for C3 fragments (9, 15, 34). This indicates that CRIg functions to enhance phagocytosis only in resident, non-inflammatory tissue macrophages, whereas CR3 functions as the dominant CR in activated macrophages and neutrophils recruited to the sites of inflammation. CRIg expression therefore associates with a subpopulation of resident macrophages that likely participate in phagocytosis of C3-opsonized particles before phlogistic events that shape the ultimate immune response. The capability of CRIg to increase phagocytosis in adRPMs and its absence from inflammatory macrophages fits well with a function in immune clearance and tissue homeostasis (35). Furthermore, CRIg has been described on synovial lining macrophages of arthritic joints but was absent from infiltrating sublining macrophages (36). Their recruitment to the site of particle contact may mediate incorporation of CRIg in the forming phagosome ensuring close contact of the macrophage plasma membrane with the opsonized particle. Indeed, Abs that blocked CRIg binding to iC3b prevented recruitment of CRIg to the phagosomes, resulting in reduced phagocytosis of the opsonized target particles (Fig. 5).

Whether the recruitment of CRIg+ endosomes to the forming phagosome is an active process is unknown. CRIg has a relative large cytoplasmic tail containing several tyrosine residues that can potentially serve as docking sites for signaling components or cytoskeletal elements. The participation of CRIg in signaling pathways linking cytoskeletal reorganization to phagocytosis is an area of current investigation.

Since in vivo tissue resident macrophages are continuously exposed to activating stimuli, including bacterial components from enteric microflora (41), we determined whether CRIg expression or phagocytic function changes in vitro following activation of adRPMs with LPS. Although cell surface expression of CRIg was slightly reduced under these circumstances, CRIg remained functional as a phagocytic receptor as shown by a similar contribution of CRIg to EAC phagocytosis in nonstimulated as well as stimulated adRPMs. Thus, while CRIg is absent from inflammatory macrophages, it remains expressed and functional when tissue resident macrophages are exposed to activating stimuli.

In a recent study (42), it was demonstrated that CRIg clears complement-opsonized platelets lacking DAF and Crry, for the first time illustrating a role of CRIg in clearance of self cells.
Unexpectedly, complement C3-coated erythrocytes were not cleared through a CRIg-dependent mechanism. A likely explanation for this finding is that complement opsonization of the DAF and Cry double-deficient erythrocytes was much less efficient compared with platelet opsonization, resulting in a significantly reduced interaction with CRIg. In the present study, IgM anti-Forsmann Ag was used to provide optimal complement activation on the erythrocyte surface leading to high levels of C3 deposition (Fig. 3A), comparable to what was found on the platelets (42). Thus, next to a potential influence of the nature of the particle, the degree of C3 opsonization is an important determinant for clearance by CRIg.

Together, CRIg identifies a novel subpopulation of macrophages with a high capacity for complement-mediated phagocytosis in the absence and presence of activating stimuli. These studies illustrate the functional diversity of tissue resident macrophages and will further guide research to understand the role of CRIg in tissue homeostasis and host defense.

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Disclosures
All authors are current or former employees of Genentech.

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