Enforced immune complex binding was independent of FCγRI surface expression levels. FCγRI, saturated with prebound IgG, was found capable of effective immune complex binding upon cytokine stimulation. Cytokine-enhanced binding was observed across a variety of immune complexes, including huIgG3- or mIgG2α-opsonized red blood cells, rituximab- or ofatumumab-opsonized B-cell lymphoma, and cetuximab-opsonized glioblastoma cells. This study contributes to our understanding of how FCγRI can participate in the clearance of opsonized particles despite saturation by monomeric IgG. (Blood. 2010;116(24):5327-5333)

Introduction

Fc receptors are receptors for immunoglobulins that play a central role in immunity. On human leukocytes, a myriad of Fcγ receptors are expressed; among these receptors, FCγRI is the only known high-affinity receptor for immunoglobulin G (IgG).1 FCγRI is constitutively expressed on monocytes, macrophages, and myeloid dendritic cells. Interferon (IFNγ) can enhance surface expression of FCγRI on these cells; and FCγRI expression can be induced on granulocytes by IFNγ or granulocyte colony–stimulating factor (G-CSF) stimulation. In contrast, cytokines such as interleukin-4 (IL-4), IL-10, and transforming growth factor-β (TGF-β) down-regulate activating Fc receptors, including FCγRI, and enhance expression of the inhibitory receptor FCγRIIB.2,3 In vitro, IFNγ treatment leads to increased Fcγ receptor–induced cytokine production.4

In vivo, the role of FCγRI in immunity remains unclear, as due to its high affinity FCγRI is believed to be saturated with monomeric IgG. This has led to the concept that prebound monomeric IgG prevents participation of FCγRI in clearing immune complexes by extravasated effector cells.5 Nevertheless, several in vivo studies documented a role for FCγRI, varying from a contribution during inflammation and autoimmune reactions,6-8 or, during monoclonal antibody (mAb)–based immunotherapy in melanoma and B-cell lymphoma models,9,10 to a malaria model in during monoclonal antibody (mAb)–based immunotherapy in melanoma and B-cell lymphoma models,9,10 to a malaria model in

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Methods

Antibodies and reagents

Anti-FCγRI-A647 was from BioLegend (clone 10.1). Unlabeled 10.1 mAb and mouse IgG1/IgG2a isotype were from eBioscience and BD Pharmin- gen, respectively. Anti-human Glycoporphin A mouse IgG2a hybridoma supernatant was described previously;24 anti-RhD human IgG3 and IgG1 (clones BRAD3 and BIRMA D6, respectively) were from the International Blood Group Reference Laboratory. Human IgG1 anti-RhD clones LHM76/58 and ESD-1 were from Alba Bioscience. Unlabeled polyclonal human IgG3 and IgG1 were purchased from Sigma-Aldrich. Okadaic acid, LY294002, and U0126 were purchased from ALEXIS Biochemicals. Monoclonal antibody IV.3 was isolated from hybridoma supernatant, and IV.3 Fab fragments were made by Fusion Antibodies.

Cell lines and monocyte isolation

Ba/F3 cells were cultured in RPMI1640 medium (GIBCO) supplemented with 10% fetal calf serum, penicillin/streptomycin, and mouse IL-3, as


The online version of this article contains a data supplement.
described. A1207 and Daudi were kept in RPMI1640 medium supplemented with 10% fetal calf serum and penicillin/streptomycin.

The retroviral vector pMX FcγRI internal ribosome entry site (IRES) green fluorescent protein (GFP) was described previously.25 Amonothropic viral particles produced in HEK293T cells were used to transduce Ba/F3 cells. After transduction, Ba/F3-FcγRII cells were sorted on a FACSaria (BD Biosciences) on green fluorescent protein expression and further subcloned by limited dilution. For all experiments where subclones are shown, results were repeated with a polyclonal line and/or with at least 2 subclones.

Primary monocytes were from healthy donors. Magnetic-activated cell sorting (MACS)–isolated monocytes were obtained from peripheral blood mononuclear cells (PBMCs) using CD14-beads (Miltenyi Biotec).

Red blood cells (RBCs) were from ficoll-separated blood. RBCs were kept in Alsever buffer (Sigma-Aldrich) for a maximum of 1 week at 4°C.

Monomeric IgG binding

Ba/F3-FcγRII cells were stimulated as described in “FACS-based EA rosette assay.” Next, cells were washed and incubated with various amounts of mouse IgG2a or huIgG3. After a 1-hour incubation on ice, cells were washed and stained with goat F(ab’)2 anti–mouse IgG (or anti–human IgG) phycoerythrin (PE; SouthernBiotech). After washing, cells were fixed with 1% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and analyzed on a FACS Canto II.

FACS–based EA rosette assay

Fluorescence-activated cell sorting (FACS)–based erythrocytes coated with antibody (EA) rosette assay was adapted from Beekman et al.26 Briefly, Ba/F3-FcγRII cells were starved overnight in RPMI1640 containing 1% fetal calf serum (FCS; RPMI 1% FCS). The next day, cells were stimulated for various time points with 30 ng/mL recombinant mouse IL-3 (PeproTech) in RPMI 1% FCS or in RPMI 1% FCS alone (medium control). RBCs were fluorescently labeled with PKH26 (Sigma-Aldrich) according to the manufacturer’s protocol, opsonized on ice with mIgG2a anti-glycophorin or huIgG3 anti–Rhesus D, and subsequently washed and resuspended in RPMI EDTA (ethylenediaminetetraacetic acid; RPMI1640, 1% FCS, 10mM huIgG3 anti–Rhesus D, and subsequently washed and resuspended in RPMI 1% FCS or in RPMI 1% FCS alone (medium control). RBCs were fluorescently labeled with PKH26 (Sigma-Aldrich) according to the manufacturer’s protocol, opsonized on ice with mIgG2a anti-glycophorin or huIgG3 anti–Rhesus D, and subsequently washed and resuspended in RPMI EDTA (ethylenediaminetetraacetic acid; RPMI1640, 1% FCS, 10mM EDTA). In a 96-well plate, 1 × 10^5 Ba/F3 cells/well were combined with 1 × 10^5 RBCs/well in RPMI EDTA and incubated for 1 hour at 4°C on a shaker. Cells were washed once with cold PBS and resuspended in cold PBS containing 1% PFA. Rosetting was analyzed on a FACS Canto II (BD Biosciences). It was shown previously that bovine IgG does not bind to human FcγRI.27

Blocking mAb 10.1 or isotype control was added at 20 µg/mL to Ba/F3-FcγRII cells before rosette assay and were kept present during assay at 10 µg/mL.

Rosette assays using IgG coupled to tosyl-activated Dynabeads was adapted from Bracke et al.28 Briefly, human IgG1 was coupled to tosyl-activated Dynabeads-M450 (Invitrogen) as described by the manufacturer. IgG-coupled dynabeads were then added to Ba/F3-FcγRII cells in RPMI EDTA in a ratio of 3.5 beads/cell. Dynabeads were found sufficiently autofluorescent in the PE channel to discriminate between Ba/F3 cells, beads, and Ba/F3 cell–bound beads. Rosettes were visualized on a LSM710 confocal microscope (Zeiss) equipped with a transmission photomultiplier tube for differential interference contrast (DIC) imaging. Pinhole opening was set to maximum.

For competition assays with monomeric ligand, Ba/F3-FcγRII cells were incubated with various concentrations of polyclonal huIgG3 on ice (Sigma-Aldrich). Unbound IgG was removed by washing Ba/F3-FcγRII cells before EA rosette assay. For inhibition experiments, Ba/F3- FcγRII cells were treated for 30 minutes with dimethyl sulfoxide (DMSO), 20µM LY294002, 20µM U-0126, or 1µM okadaic acid (OA) and stimulated with IL-3 in the presence of the inhibitor. Subsequently, cells were washed and subjected to EA rosette assay.

For measuring binding to tumor cells, tumor cell lines were fluorescently labeled with Lavacell (Activemotif), an amino reactive dye, and subsequently opsonized with 0.25 µg/mL rituximab (Roche) or ofatumumab (Genmab/GlaxoSmithKline) for Daudi or cetuximab (Merck) for A1207. After washing, the opsonized cells were incubated with Ba/F3-FcγRII for 1 hour at 4°C on a shaker. Cells were washed once with cold PBS and fixed in PBS containing 1% PFA. Binding was measured on a FACSCanto II and visualized on a LSM710 confocal microscope (Zeiss) equipped with a transmission photomultiplier tube for differential interference contrast imaging. Pinhole opening was set to maximum.

Binding assay with primary monocytes

For inhibition of inside-out signaling, monocytes were isolated as described in the previous “Cell lines and monocyte isolation” paragraph. After overnight incubation with 400 U/mL IFNγ, monocytes were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) or CellTracker Violet (Invitrogen) and treated with OA for 30 minutes at 37°C. Monocytes were plated out at 1 × 10^6 cells per well in RPMI EDTA containing 5 µg/mL IV3 antigen-binding fragments (Fabs). After 10 minutes incubation on ice, 1 × 10^6 opsonized RBCs were added. Cells were allowed to bind for 1 hour at 4°C on a shaker. After fixation in 1% PFA, binding was analyzed on a FACSCanto II.

For short-term stimulation with interleukins, PBMCs were isolated using ficoll. Cells were allowed to rest for 1 hour in 1% FCS RPMI at 37°C. Next, PBMCs were stimulated with either IL-4 (500 U/mL), IL-6 (200 U/mL), or tumor necrosis factor (TNFα) and IFNγ (500 and 400 U/mL, respectively) for 1 hour in 1% FCS RPMI at 37°C. Subsequently, PBMCs were allowed to bind to PKH26-labeled opsonized erythrocytes for 1 hour at 4°C in a 96-well V-bottom plate. After incubation, the plate was spun down and CD14-fluorescein isothiocyanate (FITC) in PBS with 5% normal mouse serum (NMS) was added to each well. The amount of monocyte binding to RBCs was scored as the percentage of CD14, PKH26 double positive events.

Statistical analysis

Data analysis was performed using Microsoft Excel 2007 and GraphPad Prism 4 software. Sigmoidal dose-response curves were used to fit rosette-binding curves data. Fitted data were tested using the F-test (comparing curves between treatments). Blocking experiments were tested using unpaired Student t test. Error bars depict SDs.

Results

IL-3 stimulation does not affect surface expression of FcγRI on Ba/F3-FcγRII cells

To determine whether FcγRI is regulated inside out, we used the IL-3–dependent Ba/F3 model. Parental Ba/F3 cells do not express any endogenous FcγR receptors but do express the FcγY chain (Ho et al.29 and supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). One-hour IL-3-stimulation of an FcγRI-transduced Ba/F3 subclone (Ba/F3-FcγRI) did not affect FcγRI surface expression levels (Figure 1A). Similar results were obtained in a polyclonal line (data not shown). These findings allowed us to perform ligand-binding studies independent of changes in receptor surface expression.

Monomeric IgG binding is a unique characteristic of FcγRI. IL-3–treated Ba/F3-FcγRI cells showed a modest, albeit significant, increased binding to mouse IgG2a (mIgG2a), human IgG1, and human IgG3 (huIgG3; Figures 1B–D, respectively). These data demonstrate that short-term stimulation with IL-3 mildly affects monomeric IgG binding in the absence of effects on receptor surface expression levels.

Inside-out regulation of FcγRI enhances immune complex binding

To investigate whether treatment of Ba/F3-FcγRI cells with IL-3–altered immune complex (IC) binding, we used a FACS-based EA rosette assay. This method was previously shown to
Furthermore, IL-3 stimulation led to a marked increase in binding of 2 or more opsonized RBCs to Ba/F3-FcγRI (Figure 2E). Binding to huIgG3-IC was FcγRI-dependent as determined by receptor blocking with FcγRI mAb 10.1 (data not shown).

We tested several IgG1 RhD clones (BIRMA D6, ESD-1, and LHM76/58) in the rosette assay. Despite considerable opsonization, IgG1 opsonized RBCs failed to bind to Ba/F3-FcγRI or primary monocytes (data not shown). This is in concordance with previous reports. It was suggested that in contrast to IgG1, the longer hinge region of IgG3 is capable of overcoming the forces between the negatively charged RBC and the effector cell. Therefore, we used IgG1-coupled beads (huIgG1-IC) to evaluate cytokine-enhanced binding of huIgG1-IC to Ba/F3-FcγRI. IgG1-coupled beads bound to Ba/F3-FcγRI cells, whereas bovine serum albumin (BSA)-coupled beads did not. IL-3 stimulation enhanced only binding of IgG1-coated beads, and binding was FcγRI-dependent, as blocking mAb 10.1 decreased binding to near background levels (Figure 2F). The formation of rosettes with IgG1-coupled beads and Ba/F3-FcγRI cells could clearly be observed with fluorescence microscopy (Figure 2G).

We next assessed whether FcγRI was similarly regulated in primary monocytes and tested the effect of several proinflammatory cytokines on IC binding. Stimulation with TNFα and IFNγ, a known macrophage stimulus, enhanced IC binding by primary monocytes. IC binding was inhibited by Ab 10.1, but not isotype control, supporting the involvement of FcγRI (Figure 2H and supplemental Figure 3A). Under these conditions, monocytes were positive for surface IgG. Upon stimulation, only slightly increased surface IgG levels were detected, suggesting that TNFα and IFNγ-enhanced IC binding to monocytes was not due to a decrease of prebound IgG to FcγRI after stimulation (supplemental Figure 3B). Furthermore, stimulation did not alter FcγRI surface expression levels but often (2 of 4 donors) coincided with increased CD11b expression levels (Figure 2I and supplemental Figure 3B). The cytokines IL-6 and IL-4 did not enhance the binding of IC to FcγRI. Taken together, these data suggested cytokine stimulation increases binding of multivalent ligands (IC) to FcγRI.

Cytokine stimulation enhances FcγRI binding to opsonized tumor cells

To date, rituximab (RTX) is one of the most successful monoclonal antibodies used in the clinic. Using RTX in combination with a B-cell lymphoma line (Daudi), we studied binding of Ba/F3-FcγRI to multivalent ligands. IL-3 stimulation led to increased binding of RTX-opsonized Daudi cells to Ba/F3-FcγRI (Figure 3A). IL-3 induced similar increased binding of Ba/F3-FcγRI to Daudi cells opsonized with ofatumumab, a newly developed human CD20 mAb (Figure 3A). Binding was FcγRI-dependent, as evidenced by blocking with mAb 10.1 (supplemental Figure 4). To investigate whether this effect was limited to B-cell lymphoma and CD20 mAb, we tested the human glioblastoma cell line A1207. IL-3 stimulation increased cetuximab-opsonized A1207-binding to Ba/F3-FcγRI (Figure 3B). Binding of Ba/F3-FcγRI to opsonized Daudi or A1207 was confirmed using fluorescence microscopy (Figure 3C-D, respectively).

FcγRI inside-out regulation is inhibited by the phosphatase inhibitor, okadaic acid

IL-3 stimulation of Ba/F3 cells leads to activation of the phosphatidylinositol-3-kinase–protein kinase B (PI-3K-PKB) and Raf/
mitogen-activated protein kinase/extracellular-signal-regulated kinase (Raf/MEK/ERK) pathways. It has been shown that FcγRI inside-out regulation in Ba/F3 cells is dependent on PI-3K and protein phosphatase 2 (PP2a).\textsuperscript{17,18} We investigated whether similar signaling pathways underlie FcγRI inside-out regulation. In our model, both a PI3-Kinase inhibitor (LY294002)\textsuperscript{32} and a MEK1/2 inhibitor (U0126)\textsuperscript{33} did not affect IL-3–enhanced binding to mIgG2a-IC (Figure 4A). OA, a PP2a inhibitor,\textsuperscript{34} completely inhibited the IL-3-enhanced binding.

To investigate whether FcγRI was similarly regulated on primary monocytes, we stimulated monocytes overnight with IFNγ before OA treatment. OA efficiently inhibited binding of opsonized RBCs to monocytes in a dose-dependent manner (Figure 4B).

Importantly, treatment of OA did not affect FcγRI-expression levels (supplemental Figure 5). In these assays, binding of IC to FcγRI was prevented by the addition of FcγRII-specific IV.3 Fab before adding IC. FcγRI-dependent binding of opsonized RBC was confirmed with mAb 10.1 (Figure 4C). These data suggest that protein phosphatases play a role during FcγRI inside-out regulation.

Cytokine stimulation enhances binding of immune complexes to saturated FcγRI

Inside-out regulation of FcγRI in the Ba/F3 model mainly affected binding to multivalent ligands (immune complexes) rather than monomeric IgG. We hypothesized that this shift could contribute to immune complex binding of FcγRI already saturated by monomeric ligands.

By saturating Ba/F3-FcγRI cells with monomeric IgG3 after stimulation, we studied whether opsonized RBCs were able to bind to IgG-occupied FcγRI. In unstimulated cells, preincubation with as little as 5 \(\mu\)g/mL monomeric IgG3 blocked RBC binding to Ba/F3-FcγRI cells to near background levels. Stimulation with

\begin{figure}
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\caption{Stimulation of primary monocytes and Ba/F3-FcγRI cells results in enhanced IC binding. Percentage of Ba/F3-FcγRI cells binding RBCs opsonized with IgG2a (A–C) or human IgG3 (D–E). Open squares represent starved cells, filled squares represent starved Ba/F3-FcγRI cells stimulated with IL-3 for 1 hour. (B) EA rosette assay in the presence of FcγRII-specific mAb 10.1 (10.1) or isotype control (iso). **P < .01; NS: not significant, t test. (C) Effect of duration of IL-3 stimulus on IC binding. *P < .05; **P < .01, t test. (D) Binding of 1 or more RBCs to Ba/F3 cells. (E) Binding of 2 or more RBCs to Ba/F3 cells. Datapoints represent the means of at least 3 replicates. Similar results were obtained in 3 independent experiments. ***P < .001. (F) Binding of Ba/F3-FcγRI cells to BSA or IgG1-coupled beads in the presence of 10.1 mAb (10.1) or isotype control (iso). Similar results were obtained in 3 independent experiments. **P < .01, t test. (G) Rosetting of BSA and IgG1-coupled beads with Ba/F3-FcγRI cells visualized with fluorescence microscopy. Beads are detected in the red channel, Ba/F3-FcγRI in the green channel. (H) Binding of unopsonized (unopson) and opsonized (opson) erythrocytes to primary monocytes in the presence of 10.1 mAb (10.1) or isotype control (iso). Data represent means of triplicates measured from 1 donor. Similar results were seen in 3 of 4 donors. *P < .05; **P < .01. (I) FcγRII (CD64) and CD11b expression on CD14-gated monocytes. Shown is the fold increase in surface expression relative to unstimulated (medium) cells. Data represent mean from triplicates.
\end{figure}
IL-3, however, led Ba/F3-FcγRI to bind opsonized RBCs effectively (Figure 5A, B). Binding was lower compared with unoccupied FcγRI but quickly reached a plateau, which was maintained at saturating levels of monomeric IgG3 (20 μg/mL). At this concentration, FcγRI was saturated by IgG3 as evidenced by surface IgG staining (Figure 5C). These data document that cytokine stimulation can lead to FcγRI IC binding despite saturation of the receptor with monomeric IgG.

Discussion

The role of FcγRI in immunity remains unclear, as it is unknown how a high-affinity Fcγ receptor can bind immune complexes. Extravasating monocytes originate from an IgG rich milieu, and it is believed that due to the high affinity of FcγRI, this receptor may well be saturated with monomeric IgG. Only after de novo synthesis has FcγRI been postulated to contribute to the clearance of IgG-opsonized particles. Here, we show that FcγRI ligand–binding can be regulated inside out (Figures 2–4) and that this regulation impacts IC binding to a saturated receptor (Figure 5).

Inside-out regulation represents a fast process, as IL-3 enhanced binding could be detected within 15 minutes after stimulation (Figure 2C). Cytokine-enhanced IC binding was observed with different ICs, including tumor cell lines opsonized with IgG1–therapeutic mAbs, as well as mouse IgG2a– and human IgG3–opsonized RBCs and human IgG1–coupled beads (Figures 2–3). Increased binding induced by cytokines was not associated with differences in receptor-surface expression levels. The presence of EDTA in our binding assays makes it unlikely that integrin interactions are involved during cytokine-enhanced IC binding. Furthermore, we did not observe increased binding to unopsonized cells upon stimulation.

Increased binding of ligand upon cellular activation was particularly striking when multivalent ligands (ICs) were used (Figure 3). Monovalent ligands showed a small increase in binding (Figure 1) upon stimulation. This could be due to small aggregates in our IgG preparations, as IgG dimers formed with mouse anti–human light-F(ab')2 showed a small increase in binding upon stimulation (data not shown). Because the increase in binding was most apparent using multivalent ligands, we speculate that the main mechanism involved in regulating IC binding is likely due to alterations in receptor mobility and/or receptor clustering. Photobleach experiments with an FcγRI-YFP fusion protein suggested increased mobility of the receptor upon IL-3 stimulation in Ba/F3.
cells (Honing et al, unpublished data). On the other hand, dimerization of FcγRI has been previously suggested and FcγRI has been shown to associate with plasma membrane microdomains. Indeed, FcγRI-binding capacity could be altered upon cholesterol depletion. A model where lipid raft localization and subsequent clustering affects ligand binding has also been suggested for FcγRIIA. It is important to note that the inside-out regulation described here is probably different from the active “trapping” of IC by FcγR as suggested by Dale et al, as our binding experiments were performed at 4°C. Cytokine-enhanced ligand binding was capable of inducing IC binding despite the presence of prebound IgG to FcγRI. It is possible that the affinity of FcγRI might differ between antigen-bound (in IC) and antigen-unbound IgG (prebound IgG to the receptor). However, it seems likely that such differences would be antigen dependent.

Several mouse models have shown involvement of FcγRI during immunotherapy. We found that ligand binding by mouse FcγRI is at least differently regulated compared with human FcγRI. Ligand binding to IC by mouse bone marrow–derived dendritic cells (DCs) and bone marrow–derived macrophages was not affected by cytokine stimulation or OA treatment (supplemental Figure 5). Thus, mouse FcγRI is not capable of inside-out regulation, or else it is dependent on different stimuli. Mouse and human FcγRI both bind IgGs with high affinity; however, they show only 19% sequence similarity with the intracellular, c-terminal tail.

Previously, dephosphorylation of FcαRI was shown to regulate ligand binding and to be dependent on PP2A. Unlike FcαRI, however, serine phosphorylation of the cytosolic tail of FcγRI α-chain is unlikely to play a role during inside-out regulation, because a truncated receptor lacking all serines was found to respond to cytokine stimulation (data not shown). Strikingly, PI-3K inhibition did not affect cytokine-enhanced IC binding to FcγRI, suggesting distinct mechanisms underlying FcγRI regulation compared with FcαRI.

In earlier reports, we published the interaction between the cytosolic tail of FcγRI and periplakin to affect receptor-ligand binding. Ba/F3-FcγRI cells did not show periplakin-dependent inside-out regulation, as truncation of FcγRI and overexpression of c-terminal periplakin did not affect cytokine-enhanced ligand binding (data not shown). This could be due to low expression levels of endogenous periplakin in Ba/F3 cells.

Overall, our data suggest that FcγRI saturated with monomeric IgG is capable of binding multivalent immune complexes. This finding contributes to the Fcγ receptor field, as it suggests that FcγRI can participate in clearance of IgG-opsonized particles despite saturation with monomeric ligand. Furthermore, it opens the field for targeted immunotherapy to this receptor. FcγRI-targeted immunotherapy can be achieved via bispecific antibodies or the use of aglycosylated IgGs to bind selectively to FcγRI. Studies using a chimeric receptor consisting of carcinoembryonic antigen–specific scFv fused to the cytosolic tail of FcγRI have demonstrated the potent cytotoxic effects mediated through this receptor. Direct targeting of FcγRI by expressing scFv-H22 on tumor cell lines showed similar results.

Our data provide new insights into the role of FcγRI in biology, although the precise mechanism underlying FcγRI inside-out regulation needs to be elucidated. Furthermore, our finding that FcγRI can contribute to immune complex binding warrants further research into the efficacy and role of FcγRI during immunotherapy.

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Authorship

Contribution: C.E.v.d.P., R.A.K., and P.B. designed and performed research and analyzed and interpreted data; J.A.v.d.L. and M.B. performed research; and C.E.v.d.P., J.G.J.v.d.W., and J.H.W.L. designed research and wrote the manuscript.

Conflict-of-interest disclosure: Ofatumumab is a product of Genmab/GlaxoSmithKline and J.G.J.v.d.W. is an employee of Genmab. The remaining authors declare no competing financial interests.

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References


Cytokine-induced immune complex binding to the high-affinity IgG receptor, FcγRI, in the presence of monomeric IgG

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