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J Immunol 2011; 186:2699-2704;
doi: 10.4049/jimmunol.1003526
http://www.jimmunol.org/content/186/5/2699

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Functional Characteristics of the High Affinity IgG Receptor, FcγRI

Cees E. van der Poel,* Robbert M. Spaapen,‡ Jan G. J. van de Winkel,*‡ and Jeanette H. W. Leusen*

IgG FcRs are important mediators of immunity and play a key role during Ab-based immunotherapy. Within the leukocyte IgG receptor family, only FcγRI is capable of IgG binding with high affinity. FcγRI exists as a complex of a ligand binding α-chain and an FcγR γ-chain. The receptors’ α-chain can, furthermore, elicit several functions independent of the ITAM-bearing FcγR γ-chain. Functional implications of high-affinity IgG binding and mechanisms underlying FcγR γ-chain–independent signaling remain unclear to this day. In this paper, we provide an overview of past literature on FcγRI and address the implications of recently described interactions between cytosolic proteins and the FcγRI α-chain, as well as cytokine-enhanced FcγRI immune complex binding. Furthermore, an analysis of potential polymorphisms within the FCGRIA gene is provided. *The Journal of Immunology, 2011, 186: 2699–2704.

Antibodies are key players in immunity. The specificity and potent immune-activating properties of Abs are exploited during mAb immunotherapy. FcRs have been shown to play a significant role in clearance of tumor cells during Ab therapy (1). All 28 mAbs currently accepted for clinical use by the Food and Drug Administration of the United States are of the IgG isotype, making FcγRs a major field of interest.

The high-affinity IgG binding of FcγRI is unique within the FcγR family. This property initially sparked interest for FcγRI within the FcγR family. Over the years, however, attention shifted to other FcRs as FcγRs’ high affinity was postulated to result in saturation of the receptor by serum IgG, thus hampering binding of opsonized pathogens or tumor cells (2, 3). In the past few years, however, the number of publications addressing FcγRI has increased. In this paper, we give an overview of the current knowledge on the role of FcγRI in immunity and Ab-based therapies, including recent studies on interactions with cytosolic proteins. FcγRI as a target for Ab treatment is reviewed elsewhere (4). Furthermore, we analyzed putative single nucleotide polymorphisms (SNPs) within the FCGRIA gene and discuss their potential impact on FcγRI functioning, based on a structural model of the receptor.

The FcγR family

The FcγR family in humans is composed of activating receptors, FcγRI, FcγRIIA, FcγRIIC, and FcγRIIIA, and an inhibitory receptor, FcγRIIB (2). Activating FcγRs can induce phagocytosis, Ag presentation, the production of reactive oxygen species (ROS) and cytokines, and Ab-dependent cellular-mediated cytotoxicity.

FcγRI and FcγRIIA are expressed on most myeloid cells including monocytes, macrophages, and dendritic cells (DCs). In addition, granulocytes express FcγRI upon activation, and FcγRIIA can also be expressed on platelets and B cells. IFN-γ is a potent stimulator of FcγRI expression (5). FcγRIIB is primarily expressed on NK cells, DCs, and a subset of monocytes. FcγRIIB is also expressed on these cell types with the exception of NK cells. NK cells and monocytes and/or neutrophils express FcγRIIC mRNA, and protein expression was confirmed on NK cells (6). FcγRIIB is only expressed on neutrophils.

In mice, FcγRI has similar high-affinity binding properties as its human ortholog. Although the extracellular structures are comparable, considerable differences in intracellular domains exist between mice and humans (only 19% sequence similarity). Mice lack FcγRIIA but express FcγRIV, which shows 63% sequence similarity with human FcγRII (7). Importantly, murine FcγRI and FcγRIV preferentially bind mIgG2a, and in contrast to FcγRI, FcγRIV binds this isotype with moderate affinity (8). Mouse models underlined the role of various FcγRs in normal immunity and in various disease models and Ab therapy. Notably, based on sequence data and existence of FcγRIV, considerable differences exist between murine and human FcR families.

The FcγRα–γ-chain complex

FcγRI consists of a ligand-binding α-chain and an ITAM-bearing FcγR γ-chain. For the human α-chain, there are three genes (FCGRIA–C) located on chromosome 1 reported to

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Received for publication October 27, 2010. Accepted for publication December 7, 2010.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003526
Several studies have suggested the cytoplasmic tail of the Fc Interactors of the Fc receptor complex, leading to phosphorylation of the ITAM on the FcR γ-chain by Src kinases and subsequent recruitment of Syk (12). Several proteins, including phospholipase C, PI3K, and SH2 domain containing leukocyte protein of 76 kDa can interact with Syk to initiate further downstream signaling leading to cytoskeletal changes, ROS, and cytokine production. SH2 domain containing leukocyte protein of 76 kDa is needed for FcR-mediated ROS production in neutrophils (13), but not in macrophages (14, 15), which may be because of differential FcR expression. Linker for activation of T cells (LAT) and LAT2 can be phosphorylated by Syk and Src kinase Lyn (16, 17) and have been shown to be critically involved in myeloid ITAM signaling (18–20). It has been suggested that FcγRI, but not FcγRIIa, cross-linking leads to phosphorylation of LAT2 in the human monocytic cell line THP-1. Other studies have also shown differential signaling between FcγRI and FcγRIIa (21), although both receptors signal in a Syk-dependent manner (22, 23).

The basis for differential signaling between various FcγRs remains unknown but could be due to differences in ITAMs (FcγRIIa has a noncannonical ITAM). Most studies rely on immune complexes (ICs) to stimulate FcγRs or use cell lines that do not express FcγRs at a similar level, complicating qualitative differentiation between FcR signaling. Alternatively, differential signaling between FcγRI may be mediated by ligand-binding α-chains.

Interactors of the FcγRI ligand-binding α-chain

Several studies have suggested the cytoplasmic tail of the FcγRI α-chain (FcγRI–CY) to affect receptor functioning including receptor-induced cytokine production and Ag presentation (24–26). On the basis of these findings, our laboratory performed yeast two-hybrid screens and documented the interaction of FcγRI–CY with periplakin, 4.1G, and filamin A (27–29).

Without filamin, FcγRI relocated to a lysosomal compartment, suggesting filamin to stabilize FcγRI surface expression (28). Upon receptor cross-linking FcγRI–filamin association is lost in agreement with previous studies (30). We suggested that filamin prevents internalization, degradation, and potentially subsequent Ag presentation.

Protein 4.1G is a member of the band 4.1 family and has been implicated to play a role in membrane stabilization of glutamate receptor subunits (31, 32). However, our latest experiments do not suggest a role for 4.1G in FcγRI membrane stabilization in unstimulated cells (C. E. van der Poel and J. H. W. Leusen, unpublished observations). Alternatively, 4.1G might be involved in FcγRI signaling as 4.1G, and the highly homologous 4.1R have been implicated in T cell signaling (33, 34).

Periplakin, initially described as a constituent of desmosomes and the cornified envelope of epidermal keratinocytes, was found to decrease FcγRI ligand binding (27). It was postulated that cellular activation might induce periplakin–FcγRI interaction, thus releasing prebound monomeric IgG and making FcγRI available for IC binding. Indeed, we recently documented cellular activation by cytokines to enhance IC binding, but monomeric IgG binding was marginally affected (35). Furthermore, this cytokine-enhanced IC binding appeared periplakin independent. Periplakin may alternatively function as a signaling adaptor, because it is documented to bind to protein kinase B (36).

Overall, the identification of proteins interacting with the FcγRI–CY has added a new layer of complexity in the functioning of this receptor. Although the physiological importance and molecular mechanism of FcγRI α-chain signaling are currently unclear, it is likely that by preventing FcγRI degradation, filamin may play a crucial role in γ-chain–independent Ag presentation.

Role of FcγRI in immunity

The role of FcγRI in immunity remains unclear. Knockout mice documented a critical role for FcγRI in models of Bordetella pertussis clearance and arthus reaction (37–39). These studies, furthermore, suggested a role for FcγRI in DC Ag presentation in an in vivo model of hematologic anemia and during mlgG2a-induced anaphylaxis and Ag-induced arthritis. In the latter models, redundancy with other FcγRs, such as FcγRIII and FcγRIV, exists, which may be partially attributable to the use of mlgG2a.

It remains unclear how FcγRI contributes to clearance of preformed IC. The high-affinity nature of FcγRI has been postulated to hamper interaction with IC under serum conditions (2). Notably, the high IgG content in serum is theoretically sufficient for near saturation of even low-affinity FcγRs (Table I). Several hypotheses have been proposed to explain FcγRs‘ role in immunity: 1) because of its high affinity, FcγRI is "prearmed" with IgG and functions as a type of scavenger receptor, analogous to the high-affinity receptor for IgE, FcεRI, or as a sampling receptor of extracellular Ags; 2) local inflammation leads to cytokine production causing de novo synthesis of free FcγRI; or 3) receptor reorganization on the membrane and/or conformational changes leads to preferred binding to ICs. Although there is no direct evidence supporting the first hypothesis, macrophages may well encounter Ags or pathogens that have not been in contact with IgG yet. FcγRI is known to internalize from the plasma membrane upon binding of monomeric IgG and could as such sample a constant source of extracellular Ags (40). Indeed, non–cross-linking FcγRI-specific m22-F(ab′)2 fragments induce anti-m22 idotype titers in FcγRI transgenic but not in wild-type mice (41). Cytokine enhanced upregulation of FcγRI is well documented and leads to increased receptor surface levels within several hours (5, 42, 43). Inside-out regulation is a relatively new phenomenon with respect to FcγRs. Similar to FcγRIIA and FcεRI, cytokine stimulation leads to a fast (typically within 15–30 min) increase in ligand binding by FcγRI, independent of changes in receptor expression levels, and we have observed cytokine stimulation to induce efficient competition between IC and prebound monomeric IgG (35, 44, 45). FcγRI has been shown to reside in membrane microdomains in IFN-γ–stimulated cells, and receptor clustering may affect IC binding, as shown for...
FcγRIIA (46). Mouse macrophages were found incapable of inside-out signaling, and mice may well depend more on the moderate-affinity IgG receptor FcγRI, which does not bind monomeric IgG (7, 8). Thus, in humans, FcγRI inside-out regulation may allow FcγRI to contribute to IC binding and uptake, despite saturation by serum IgG. All three hypotheses are not mutually exclusive, and we propose that, at rest, FcγRI may facilitate sampling of extracellular Ags via its bound IgG. During inflammation, inside-out signaling may lead to initial IC binding, whereas de novo generation of free FcγRI may contribute later in the response (Fig. 1).

Role of FcγRI during immunotherapy

Single, double, and triple knockouts in mice have been instrumental in determining the role of individual FcγRI in immunotherapy models. Bevaart et al. (47) showed that Ab treatment depended critically on FcγRI in a B16F10/TA99 melanoma model. Contribution of FcγRI to Ab treatment was shown in a liver metastases model using TA99 and a model using CD20 mAb (48, 49). Treatment with human IgG1 mAbs in a malaria model was completely dependent on transgenic expression of FcγRI (50). Furthermore, bispecific Ab (BsAb) targeting FcγRI and Candida albicans protected mice from infection (51, 52).

**Table 1.** FcRs are nearly saturated with ligand at serum IgG levels

<table>
<thead>
<tr>
<th>FcR</th>
<th>IgG1 Molecular</th>
<th>IgG2 Molecular</th>
<th>IgG3 Molecular</th>
<th>IgG4 Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass (kDa)</td>
<td>Mass (kDa)</td>
<td>Mass (kDa)</td>
<td>Mass (kDa)</td>
</tr>
<tr>
<td></td>
<td>Serum g/l</td>
<td>Serum g/l</td>
<td>Serum g/l</td>
<td>Serum g/l</td>
</tr>
<tr>
<td></td>
<td>4.04 × 10⁻⁶</td>
<td>2.33 × 10⁻⁶</td>
<td>5.35 × 10⁻⁷</td>
<td>1.37 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>%sat</td>
<td>%sat</td>
<td>%sat</td>
<td>%sat</td>
</tr>
<tr>
<td>FcγRI</td>
<td>1.54 × 10⁻⁷</td>
<td>1.64 × 10⁻⁷</td>
<td>2.94 × 10⁻⁷</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>76</td>
<td>18.9</td>
</tr>
<tr>
<td>FcγRIla H131</td>
<td>1.92 × 10⁻⁸</td>
<td>99.5</td>
<td>76</td>
<td>4.76 × 10⁻⁷</td>
</tr>
<tr>
<td>FcγRIla R131</td>
<td>2.86 × 10⁻⁸</td>
<td>99.9</td>
<td>76</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>96</td>
<td>5.00 × 10⁻⁷</td>
<td>21.5</td>
</tr>
<tr>
<td>FcγRIla F158</td>
<td>8.55 × 10⁻⁸</td>
<td>97.9</td>
<td>97</td>
<td>4.00 × 10⁻⁷</td>
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<td>97</td>
<td>25.5</td>
</tr>
</tbody>
</table>

Percentage of occupied receptors (%sat) is equal to the unbound ligand concentration divided by the sum of the unbound ligand concentration and the dissociation constant (Kd). The Kd was calculated from the association constants reported previously (11). FcγRI was found not to bind to IgG2.

**FIGURE 1.** Inside-out regulation of FcγRI allows binding of ICs despite serum IgG receptor saturation. A. In unstimulated cells, saturation of FcγRI by serum IgG precludes IC binding. B. During inflammation, cytokine stimulation may induce FcγRI clustering in membrane rafts within minutes. Clustering could facilitate competition of ICs over monomeric IgG for receptor binding. On a longer timescale, cytokine stimulation induces de novo protein expression of free FcγRI molecules, which can further contribute to complex binding.

**FIGURE 2.** FcγRI structure and variations. A. Amino acid sequence divided in different domains. The five nonsynonymous SNPs are indicated in red (V39I, rs7531523; R92*, previously described stop codon (70); Q224*, rs1338887; 1301M, rs12078005; and 1387T, rs1050208, asterisks denote stop codon), the probable surface residues that facilitate IgG binding are in light blue, the interaction sites with 4.1G are underlined, and the periplakin is in green (27, 29, 73). B. Interpretation of FcγRI within a plasma membrane to illustrate the interaction and variation sites. The color codes are the same as in A, and the 4.1G binding site is in magenta. All images were created in PyMol.
against infection and induced potent anti-\textit{C. albicans} immunity in FcγRI transgenic but not in wild-type mice (51). Treatment with FcγRI and B cell lymphoma-targeted BsAb was highly effective in lymphoma eradication and induced long-term T cell immunity in FcγRI transgenic mice (52).

The use of BsAbs has shown the potential of FcγRI-targeted immunotherapy in humans. Several clinical trials used chemically cross-linked H22 F(ab')2, a humanized form of anti-FcγRI mAb M22, to tumor-specific F(ab')2 against anti-HER2/neu (53) or antipertid growth factor receptor factor (54). MDX-H210, an H22 F(ab')2 × anti-HER2/neu F(ab')2 BsAb, was capable of "arming" neutrophils and monocytes in vivo, and a trial treating prostate cancer patients with MDX-H210 in combination with GM-CSF reported encouraging results (55, 56). Other studies combining G-CSF or IFN-γ with MDX-H210 were less successful (57–59). A phase 1 trial combining H22 with anti-CD30 reported 1 complete remission, 3 partial remissions, and 4 patients with stable disease out of a total panel of 10 patients with refractory Hodgkin's lymphoma (60).

Overall, FcγRI-directed BsAbs were relatively successful, given the fact that early studies were hampered because of toxicities (54, 55), difficulties in generating true heterodimeric BsAbs when using chemical cross-linking, and BsAb short half-lives (5–17 h, dose-dependent). BsAbs may have been ahead of their time in terms of a practical therapeutic strategy; now, novel technologies such as recombinant BsAb (61) and portable minipumps to overcome half-life problems (62) may warrant renewed investigation of FcγRI-targeted BsAbs. Alternatively, α-glycosylated IgG has therapeutic potential because it selectively binds FcγRI, potentially bypassing inhibitory signaling by FcγRIIB (63).

\textit{FcγRI} polymorphisms

Polymorphisms in FcγRIIA and -IIIA can alter ligand binding, influence Rituximab treatment efficacy, and are associated with autoimmune diseases and occurrence of bacterial infections (11, 64–68).

To date, no polymorphisms have been described for FcγRI that alter receptor affinity or function. FcγRII deficiency does not appear to increase susceptibility to infections or autoimmune diseases (69). Although this suggests that FcγRII could play a redundant role in immunity, a role during mAb-based immunotherapy is unclear.

To find possible polymorphisms that could alter receptor function or expression, we aligned the three \textit{FCGR} genes and reported SNPs from the ENSEMBL database. We identified 57 candidate SNPs that do not seem to result from sequence misinterpretation because of the highly homologous \textit{FCGR} genes. Only 3 of 57 candidate SNPs are nonsynonymous (rs7531523, rs12078005, and rs1050208) (Fig. 2A). In addition to the stop codon previously described (70), an rs1338887 (Q224*) encodes for a stop codon in EC3. Frequency data for potential SNPs were not available with the exception of rs1338887 (heterozygosity in 2 of 65 European individuals).

To gain a better insight in the possible impact of the nonsynonymous SNPs on receptor function, we used the I-TASSER and HADDOCK servers to predict the structure of FcγRI (Fig. 2B) (71, 72). We were able to model all three EC domains with acceptable C scores (EC1+EC2: 0.74; EC3: −0.25). EC1 and EC2 appeared similar to predictions reported earlier (73). Structural predictions of the transmembrane (TM) domain and intracellular tail were less reliable (C scores −3.49 and −2.06, respectively).

The only SNP located in the extracellular domain (rs7531523, encoding V39I) is largely buried within the structure of EC1 and does not locate within the expected IgG binding site (Fig. 2). However, V39 appears to be highly conserved within the FcγR family implying possible necessity of this amino acid in structure/function (data not shown). The I301M change (rs12078005) in the TM may be rather conserved as both residues are hydrophobic, although the TM of FcγRI has been shown to play a role in surface expression (74). The I338T mutation (rs1050208) appears to be located outside of the expected binding sites for protein 4.1G and perilipin (Fig. 1A, 1D). These data show the relative ease by which an in silico approach may help to gain further insights in the effects of nonsynonymous SNPs, although further analyses are needed to understand if and how these potential SNPs affect receptor functioning.

In recent years, copy number variations (CNV) have gained more interest in the FcγR field (reviewed in Ref. 75), and CNVs of FCGR3B and FCGR2C have been linked to increased susceptibility of autoimmune diseases (6, 76). CNVs for the \textit{FCGRIA} gene have not been reported.

\textbf{Conclusions}

Despite intense study, the biological role of FcγRI remains unclear. Nevertheless, given the receptors' potent cytotoxic effects and its capability to efficiently mediate Ag presentation, FcγRI may well play a role in mAb-based immunotherapy. Recent data from our laboratory suggest inside-out signaling to possibly overcome some of the competition by serum IgG during binding of opsonized targets. This may open up new avenues of FcγRI-mediated immunotherapy, especially because the documentation of FcγRI selectively binding α-glycosylated IgG. Otherwise, BsAbs may well be used to circumvent FcγRI ligand binding sites. One of the goals of Ab therapy should be to induce long-term memory against tumor or pathogen-derived Ags. FcγRI may be capable of mediating such effects (52). It should be investigated whether circulating monocytes become armed with therapeutic Abs during mAb therapy. Finally, our computational studies suggest at least seven putative polymorphisms to exist in the \textit{FCGRIA} gene. Experimental studies on these polymorphisms and/or CNV may provide clues to the exact role of FcγRI in immunity and Ab therapy.

\textbf{Disclosures}

The authors have no financial conflicts of interest.

\textbf{References}


