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# Immunoglobulin G1 binding with various molecular receptors: A new paradigm of IgG1 as a potential adjuvant

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ABSTRACT

**Objective**: To explore the possible IgG1 binding receptors by protein-protein docking experiments. **Methods**: The protein-protein cognate interactions such as IgG with Fc Receptors (FcRs) potentiate signaling cascades to ameliorate antigen uptake, processing and presentation are studied by protein-protein docking experiments. **Results**: However, the propensity of IgG interactions with other cognate receptors largely remains obscure. In this direction, possibilities of IgG1 binding with various five receptors were explored. In this study, we report previously unidentified associations between IgG1 and other receptors. Herein, we show that IgG1 binds to the granulocyte-macrophage receptor, β common receptor and complementary receptor(complementary receptor I and complementary receptor II) to form a complex structure. We show the binding ability and important protein-protein interactions of IgG1 with four receptors in comparison to Fc Receptor, and also find out the conserved amino acids and hydrophobic-hydrophobic interactions amongst them. **Conclusions**: Comparative interaction studies of IgG1 binding to various receptors revealed close similarities of IgG1 binding to its native receptor Fc. In conclusion, our study has shown the comparable binding efficiency of four receptors to IgG1 apart from the conventional Fc receptor.

## 1. Introduction

Immunoglobulins (Igs), which are known as antibodies, are glycoprotein molecules produced by plasma cells. They play a very important role in the immune system by specifically recognizing and binding to particular antigens, such as bacteria or viruses, and aiding in their destruction. There are five types of Igs; IgM, IgG, IgA, IgD and IgE of which IgG is the most abundant class (80%) of antibodies circulated in the serum in response to antigenic stimulus specifically. IgG is further classified into subclasses IgG1, IgG2, IgG3 and IgG4[1,2]. The extent and degree of differential Igs centered downstream immune responses show significant variability, which largely depends upon the classes and subclasses of Igs involved in signaling. IgG1 and IgG3 are more potent Igs than IgG2 and IgG4, the latter may exhibit immunological function in specific scenarios, wherein, they are capable of neutralizing virus particles and toxins[3,4].

Fragment antigen-binding(Fab) and fragment crystallizable(Fc) domain of antibodies are determinants of differential mode of action of therapeutic antibodies. Fab-domain or direct effects exert

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biological effects such as inhibition of ligand binding, apoptosis and receptor internalization. Fc-domain or indirect effects are exemplified by complement dependent cytotoxicity, antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosisadcp[5-7]. The Fc domain of the Igs interacts with Fc receptors (FcRs) found on various immune effector cells such as monocytes, macrophages, B cells *etc*., and thereby exhibit pleiotropic effects ranging from regulation of Igs' serum half-life in particular IgG to their cellular transport in general. The Fc-FcR interactions have been exploited in thedevelopment of tailored strategies in synthetic designs of different immunotherapeutics, targeted drug delivery, adjuvanted DNA vaccines. The cellular receptors for IgG belong to Fcγ receptor (FcγR) family. There are four classes of FcγRs; FcγRI (CD64), Fcγ RII (CD32), FcγRIII (CD16), and FcγRIV (CD16.2) that comprise immunoglobulin superfamily[8-12]. It has been reported that FcRs are accessory subunits that modulate signaling[13] and ligand affinity[14]. FcRs significantly shares homology with B cell receptor (BCR) and T cell receptor, harbouring a common motif, the immune receptor tyrosine activation motif, which actively participates in the recruitment and activation of specific tyrosine kinase upon cross-linking of receptors[15-17].

The granulocyte macrophage colony stimulating factor (GM-CSF) receptor, is a heterodimer, which consists of a major binding subunit granulocyte-macrophage receptor α (GMR α ) and a major signaling subunit ( β c) that is co-expressed on the surface of leukocytes. The β c receptor subunit expressed at lower levels than GMR α subunit[18-20]. The closely related IL-3 and IL-5 receptors (IL-3R and IL-5R) also use a ligand-specific α -chain and share β c with the GM-CSF receptor which is also known as BCR. The analogous nature (shared signaling subunit) of GM-CSF receptor system with the IL-6, IL-4/IL-13, and IL-2 receptor systems suggest an evolutionary conserved structural and functional arrangement. Whereby, a single polypeptide receptor chain can recognize more than one cytokine to mediate multiple biological activities. The GM-CSF receptor can signal and govern various cellular functions which include protection from entry, cell cycle control and apoptosis, early commitment to myelopoiesis, differentiation/maturation of committed progenitors, and multiple activation and motility functions in mature cells[21-23].

Human complement receptor type1 [complementary receptor I (CR1)/CD35 or C3b/C4b] is a single chain transmembrane glycoprotein which is a member of the regulator of complement activation protein family. CR1 is highly expressed (~20 000-40 000/cell) on B cells, monocytes and peripheral blood cells in addition to follicular dendritic cells (FDCs) in germinal centers of lymph node[24]. Similar to CR1, complement receptor type 2 [complementary receptor II (CR2)/CD21] a multifunctional receptor is also expressed on B cells, FDCs and a subset of peripheral and thymic T cells[25,26]. Functionally, the CR1 and CR2 receptors mediate antigen capture for B cells activation and subsequent BCR mediated signal transduction in several pathways such as actin polymerization[27], NF- κ B activation[28], antigen uptake and presentation to T cells[29,30], homotypic adhesion[31] and IL-6 generation[32].

Antigen along with molecular adjuvant that can bind to receptors like FcRs, GMR, BCR, CR1 and CR2 present on various antigen presenting cells and effector cells generate strong immune response than antigen solely. Like FcRs, other four receptors also show a similar type of binding efficiency with IgG1. In a previous study from our group a comparative study of the binding efficiency of molecular adjuvants Immunoglobulin-G1 fragment constant region (IgG1Fc), GM-CSF and complement protein 3d with complement receptor1[33] has been performed, where IgG1 showed the best binding efficiency with CR1. In continuation of the previous work and its finding, in the current study a comparative analysis was done to explore more about IgG1 and its binding to GMR, BCR, CR1 and CR2 in comparison with FcR. The outcome of the docking study revealed that binding energy of above-mentioned IgG1 and receptors lies in a relatively comparable range, which suggests IgG1 also binds with GMR, BCR and CR1 similar to FcR. Multiple sequence alignment analysis was performed for aforesaid receptors. Hydrophobic-hydrophobic residue interactions at the interface which plays a pivotal role in determining the binding of protein-protein complexes were found conserved in all the five complexes.

## 2. Materials and methods

### 2.1. Dataset

In order to make a comparative study on the binding of a molecular adjuvant with different receptors, sequences and structures of a molecular adjuvant (IgG1) and five receptors, CR1, CR2, FcR, GMR and β common receptor were obtained from Protein Data Bank, Berman *et al*. The four-letter identifier of the adjuvant and receptors along with chain identifier in the Protein Data Bank, resolution and length of the polypeptide chain is shown in Table 1. The sequences of the receptors were subjected to multiple sequence alignment by using Multialign program[34] to find out the conserved amino acid residues across the receptors. We have neglected sequence of receptors which has unknown amino acids (like x) for performing multiple sequence alignment. Multialign program incorporates an algorithm based on conventional dynamic programming and hierarchial clustering to produce accurate alignments.

**Table 1**

Details of adjuvant and receptors used in the study.

| Adjuvants (A)/Receptor (R) | PDB ID_Chain ID | Resolution (Å) | Length |
|---|---|---|---|
| IgG1Fc (A) | 3DO3_A | 2.5 | 212 |
| CR1 (R) | 1GKG_A, 5FO9_C | NMR, 3.3 | 136, 196 |
| CR2 (R) | 1LY2_A | 1.80 | 130 |
| Fc (R) | 4W4O_C, 4ZNE_A | 1.80, 2.42 | 280, 267 |
| GMR (R) | 4NKQ_A, B | 3.3 | 414, 305 |
| BCR (R) | 2GYS_A | 2.7 | 419 |

### 2.2. Docking and mutation experiments

To study the binding efficiency and to identify the important amino acid residues responsible for the adjuvant property of molecular adjuvant (IgG1) interacting with five receptors, ClusPro docking program was used[35]. ClusPro uses a method called PIPER that uses a pairwise interaction potential as part of its scoring function. ClusPro docking has shown better performance on a number of protein-protein complexes that were used as targets in the Critical Assessment of *P* Redictions of Interactions experiment[36]. The experiment was designed to test protein docking algorithms in blind predictions of the structure of protein-protein complexes. In order to support the results of ClusPro program, we used HEX protein-protein docking program which is based on the Fourier transform method to derive a top consensus binding partner[37]. Both docking programs generated ten conformation models and the top-ranked protein-protein complex model was considered for further study.

The hydrophobic-hydrophobic interactions between adjuvant and receptors were further analyzedby using a protein interactions calculation server[38]. The amino acid residues like Ala, Val, Leu, Ile, Met, Phe, Trp, Pro and Tyr were considered as hydrophobic residues and the distance cut-off used to calculate interaction is below 8Å range[39-41].

## 3. Results

Several previous studies have highlighted the functional complementarity of antibodies and complement proteins in experimental animal models as well as in human immune system[42-45]. CR1/2 are CR2 gene derived protein expressed primarily on B cells and FDCs. Compromise in antibody responses has been previously reported in a receptor CR1/CR2 knockout mouse model[46,47]. In this context, we sought to explore interactions between IgG1 and five receptors by using various computational and bioinformatics approaches. Multiple sequence alignment between five receptors was performed to discern similarities and differences at the genomic level (Figure 1). The analysis revealed that the sequence length of CR1 (136aa) and CR2 (130aa) were almost similar, whereas the length of the other three receptors (280aa, 414aa, 419aa) are higher than CR1/2. We further screened for conserved amino acids across all five sequences and found that 40 amino acid residues at various positions were conserved in CR1 and CR2 sequences as shown in multiple sequence alignment analysis results in Figure 2. The consensus conserved residues in the sequences of five receptors are indicated in blue color.

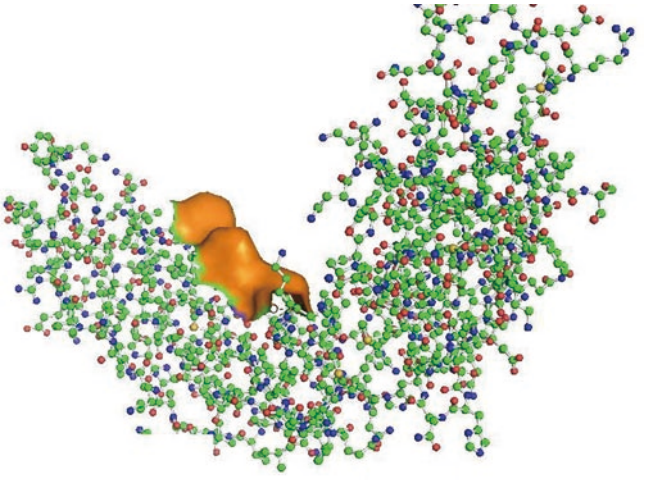

**Figure 2.** Three-dimensional structure of IgG1, important three-dimensional binding site residues conserved in all five complexes are shown as yellow surface model.
Non-binding residues are shown as ball & amp; stick model. White for hydrogen; Black for carbon; Blue for nitrogen; Red for oxygen; Deep yellow for sulfu and Purple for phosphorus;

In order to gain insights into folding and binding of IgG1 with

```
           1       10        20        30        40        50        60        70        80        90       100       110       120       130
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
GMR        EETIPLQTLRCYNDYTSHITCRWADTQDAQRLVNVTLIRRVNEDLL-EPVSCDLSDDMPWSACPHPRCVPRRCVIPCQSFVVTDVDYFSFQPDRPLGTRLTVTLTQHVQPPEPRDLQISTDQDHFLLTWS
BCR        EETIPLQTLRCYNDYTSHITCRWADTQDAQRLVNVTLIRRVNEDLL-EPVSCDLSDDMPWSACPHPRCVPRRCVIPCQSFVVTDVDYFSFQPDRPLGTRLTVTLTQHVQPPEPRDLQISTDQDHFLLTWS
FcR                                                                                                                                  QVDTT
CR1              GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSS-PKDVCKRKSCKTPPDPVN----GMVHVITDIQVGSRINYSCTTGHRLIGHSSAECILSGNTAHWSTK
CR2                                                                                                  EASCGSPPPILNG---RISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDK
Consensus  ..........................................................................sc..pp...n.........t.i.vgt.i.ysc....rlig..sl.cit.........k

           131     140       150       160       170       180       190       200       210       220       230       240       250       260
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
GMR        VALGSPQSHWLSPGDLEFEVYYKRLQDSWEDAAI--LLSNTSQATLGPEHLMPSSTYVARVRTRLAPGSRLSGRPSKWSPEVCWDSQPGDEAQPQNLECFFDGAAVLSCSWEVRKEVASSVSFGLFYKPS
BCR        VALGSPQSHWLSPGDLEFEVYYKRLQDSWEDAAI--LLSNTSQATLGPEHLMPSSTYVARVRTRLAPGSRLSGRPSKWSPEVCWDSQPGDEAQPQNLECFFDGAAVLSCSWEVRKEVASSVSFGLFYKPS
FcR        KAVITLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYRITSASVNDSGEYRCQRG--LSGRSDPIQLEI----HRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNV---LYYRNG
CR1        PPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVGEPSIYCTSNDDQV--GIWSGPAPQCIIPN
CR2        PA---PKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANNMWGPTRLPTCVSI
Consensus  pa...p.............e.i........gs.......s.t......fs..g..s..c..n......g.....................................................................

           261     270       280       290       300       310       320       330       340       350       360       370       380       390
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
GMR        PDAG------EEECSPVLREGLGSLHTRHHCQIPVPDPATHGQYIVSVQPRRAEKHIKSSVNIQMAPPSLQVTKDGDSYSLRWETMKMRYEHIDHTFEIQYRKDTATWKDSKTETLQNAHSMALPALEPS
BCR        PDAGSAVLLREEECSPVLREGLGSLHTRHHCQIPVPDPATHGQYIVSVQPRRAEKHIKSSVNIQMAPPSLQVTKDGDSYSLRWETMKMRYEHIDHTFEIQYRKDTATWKDSKTETLQNAHSMALPALEPS
FcR        KAFK---FFHWNSNLTILKTNISHNGT-YHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTLSCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDG
CR1
CR2
Consensus  ..........................................................................................................................................

           391     400       410       420423
           |--------+---------+---------+--|
GMR        TRYWARVRVRTSRTGYNGIWSEWSEARSWDTES
BCR        TRYWARVRVRTSRTGYNGIWSEWSEARSWDTE
FcR        NVLKRSPELELQVLG
CR1
CR2
Consensus  ..............................
```

**Figure 1.** Multiple sequence alignment of GMR, BCR, FcR, CR1 and CR2 receptors. Sequence position is shown in the first line and consensus of the alignment is provided in the last line of the alignment.

receptors, important hydrophobic interactions between protein-protein complex (IgG1 versus five receptors) docking simulations were undertaken. ClusPro tool was used to dock IgG1 with GMR, BCR, Fc, CR1, and CR2, to study the assembly of the complex structure formed thereof. The top 10 ranked docked complex structures were selected based on their binding energies and structures with least binding energy were chosen for further analysis as shown in Table 2. The ClusPro analysis revealed minimum binding energies of the protein-protein complexes; IgG1-GMR (-995.9 Kcal/mol), IgG1-BCR (-973.5 Kcal/mol), IgG1-Fc (-899.1 Kcal/mol), IgG1-CR1 (-813.6 Kcal/mol) and IgG1-CR2 (-776.2 Kcal/mol). It should be noted that IgG1complexed with Fc is solved experimentally by x-ray crystallography and hence we use this docking scores as a control. The HEX docking program was used to confirm our findings as obtained by ClusPro. HEX analysis showed similar binding energy trends for the entire five complexes trend as observed in our ClusPro. Based on our ClusPro and HEX findings, the binding energies of the complexes IgG1Fc-FcR and IgG1Fc-CR1/CR2 lies in the similar range, suggesting the likelihood of CR1 leading to the formation of very strong and stable complexes similar to IgG1-FcR (Table 2). The top-ranked protein-protein complexes obtained from the ClusPro program are shown in Figure 3.

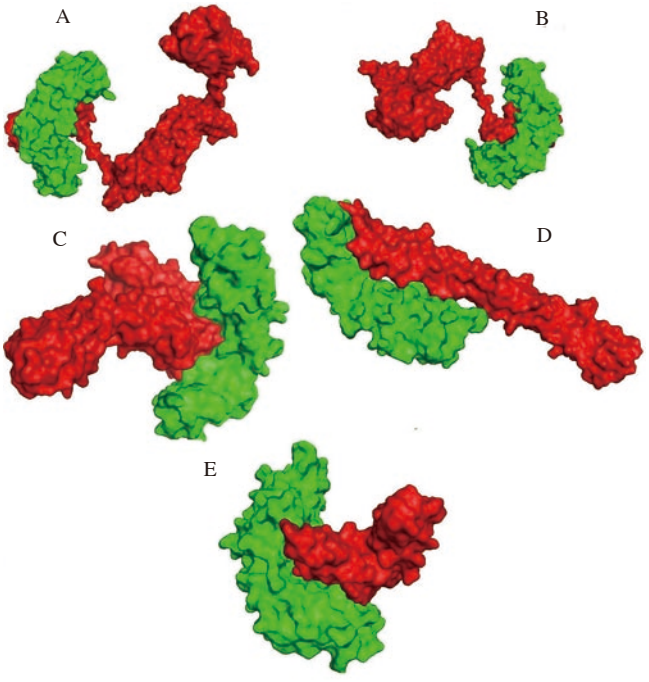


**Figure 3.** Three-dimensional structures of protein-protein complexes generated by ClusPro docking program. IgG1Fc is shown as green surface model and GMR(A), BCR(B), FcR(C), CR1(D) and CR2 (E) are shown as red surface model.

Hydrophobic interactions at the interface are very crucial for the stability of protein-protein complexes. Therefore, the number of hydrophobic residues and their interactions at the interface of protein-protein complexes were analyzed. Our evaluation showed the differential number of hydrophobic interactions at the complex interfaces; 11, 21 and 15 in IgG1-Fc, IgG1-CR1 and IgG1-CR2 respectively (Table 2). IgG1-GMR and IgG1-BCR form 18 hydrophobic interactions at the protein-protein interface. The number of hydrogen bonds formed at the protein-protein interface is shown in Table 2. The number of hydrogen bonds is computed by counting main chain-main chain, main chain-side chain and side chain-side chain hydrogen bonds at the protein-protein interface. Interestingly, IgG1-Fc receptor (Control) and IgG1-BCR form more number of hydrogen bonds (23 H bonds) than IgG1-CR1 (20 H bonds), IgG1-CR2 (18 H bonds) and IgG1-GMR (15 H bonds) respectively. It should be noted that the top ranking IgG1-GMR complex has less number of hydrogen bonds. The number of ionic, aromatic and cation-pi interactions is shown in Table 2. Three cation-pi interactions, which are believed as stabilizing interactions are formed in IgG1-Fc complex, whereas no such interactions are observed in IgG1-GMR/BCR. There is one cation-pi interaction formed in IgG1-CR1/CR2 complexes.

While observing the protein-protein interactions of IgG1 with five complexes, it is possible to find certain amino acid residues in IgG1 which are important for binding with receptors. The hydrophobic amino acid residues like 241PHE, 243PHE, 244PRO, 336ILE and 398LEU are forming hydrophobic interactions with the receptors. Similarly, the amino acid residue 376ASP form ionic interaction and amino acid residue 404PHE form aromatic interactions in all the five protein-protein complexes. The amino acid residues in IgG1 like 241PHE, 243PHE and 244PRO form hydrophobic interactions with all five receptors and these important conserved hydrophobic interactions in IgG1-GMR are shown in figure 4 along with its distance. The hydrophobic, hydrogen bonding, ionic, aromatic and cation-pi interactions in protein-protein complexes are given in supplementary material. A short hydrophobic stretch at amino terminal (FXFP) of IgG1 is important for receptor binding and we believe that these amino acid residues may play a vital role in IgG1 for its adjuvant property.

**Table 2**
Docking scores and various types of protein-protein interactions formed between molecular adjuvant and five receptors

| Complex (Adjuvant-Receptor) | Binding Energy (Kcal/mol) ClusPro | Binding Energy (Kcal/mol) HEX | Hydrophobic interaction | Hydrogen bonds | Ionic interactions | Aromatic interactions | Cation-pi interactions |
|---|---|---|---|---|---|---|---|
| IgG1-GMR | -995.9 | -1045.5 | 18 | 15 | 3 | 2 | 0 |
| IgG1-BCR | -973.5 | 997.6 | 18 | 23 | 4 | 2 | 0 |
| IgG1-FcR | -899.1 | -946.8 | 11 | 23 | 3 | 3 | 3 |
| IgG1-CR1 | -813.6 | -758.3 | 21 | 20 | 2 | 1 | 1 |
| IgG1-CR2 | -776.2 | -700.5 | 15 | 18 | 2 | 1 | 1 |

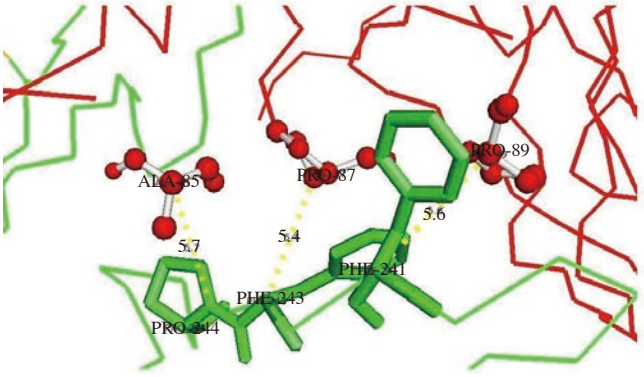


**Figure 4.** Important conserved contacts in IgG1 (green)-GMR (red) complex is shown with labels.

## 4. Discussion

IgG1 is known for its adjuvant properties and serum half-life enhancing activities. Interaction studies of IgG1Fc with GMR, BCR, FcR, CR1 and CR2 opened new possibilities of its mode of action as a potential molecular adjuvant. Even though there is a difference in length of the amino acid sequence of FcR (280aa) when compared to CR1 (136aa) and CR2 (130aa), still the binding energy of protein-protein complexes is relatively close which suggest that GMR and BCR can also make a very strong and stable complex with IgG1 like that of FcR. There is only difference of six amino acids in CR1 and CR2 common precursor derived proteins[48]. CR1 binding energy is -813.6 Kcal/mol while CR2 binding energy is -776.2 Kcal/mol, the possibility behind the drop (-37.4 Kcal/mol) in binding energy is may be due to the pivotal role of these six amino acids which might be very crucial for interactions. During the course of evolution, many related proteins even after diverging enormously at the genomic level still maintain their three-dimensional structure and interacting interface. Although there is a difference in protein length, they have a binding energy in a very close range, which suggests that, these receptor structures are evolutionary conserved. Conserved FXFP hydrophobic interactions in all the five protein-protein complexes also support this idea. Binding energy and conserved surface interactions reveal that IgG1 being an adjuvant can also target cells having GMR, BCR, CR1 and CR2 receptors, which opens new ways to explore more about the mode of action and signaling cascade triggered by IgG1Fc as a potential molecular adjuvant. Previously, our study on the binding efficiency of various adjuvants like GM-CSF, complement protein 3d and IgGFc with CR1, showed that the adjuvant IgGFc had the best binding efficiency. As a result of IgG1Fc binding to FcR, GMR, BCR, CR1 and CR2 altogether, more number of cells and different cell population could be targeted and also cells expressing more than one type of these receptors of the signaling cascade triggering may lead to signal amplification. Based on the above observations, it is very much clear that IgGFc with its receptor binding efficiency may also have an efficient molecular adjuvant activity. Due to signal crosstalk and also independent signaling, the microenvironment of the cells participating in the host-pathogen interactions may be influenced. Further, confirming the above results with the wet lab studies will help to prove the multifunctional role of IgG1 as an adjuvant, as a serum half-life enhancer and also as a key effector for the host-pathogen interactions.

## Conflict of interest statement

The authors report no conflict of interest.

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