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Short communication

In vivo effector functions of high-affinity mouse IgG receptor FcγRI in disease and therapy models

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ABSTRACT

Two activating mouse IgG receptors (FcγRs) have the ability to bind monomeric IgG, the high-affinity mouse FcγRI and FcγRIV. Despite high circulating levels of IgG, reports using FcγRI$^{-/-}$ or FcγRIV$^{-/-}$ mice or FcγRIV-blocking antibodies implicate these receptors in IgG-induced disease severity or therapeutic Ab efficacy. From these studies, however, one cannot conclude on the effector capabilities of a given receptor, because different activating FcγRs possess redundant properties *in vivo*, and cooperation between FcγRs may occur, or priming phenomena. To help resolve these uncertainties, we used mice expressing only FcγRI to determine its intrinsic properties *in vivo*. FcγRI^{only} mice were sensitive to IgG-induced autoimmune thrombocytopenia and anti-CD20 and anti-tumour immunotherapy, but resistant to IgG-induced autoimmune arthritis, anaphylaxis and airway inflammation. Our results show that the *in vivo* roles of FcγRI are more restricted than initially reported using FcγRI$^{-/-}$ mice, but confirm effector capabilities for this high-affinity IgG receptor *in vivo*.

1. Introduction

IgG receptors (FcγR) in both humans and mice are divided into high-affinity IgG receptors that are able to retain monomeric IgG, and low-affinity IgG receptors that do not. Both high- and low-affinity FcγRs are, however, able to bind to IgG-immune complexes or IgG-opsonised cells and surfaces. In humans only hFcγRI is a high-affinity IgG receptor, for human IgG1, IgG3 and IgG4; and in mice both mFcγRI (for mouse IgG2a) and mFcγRIV (for mouse IgG2a and IgG2b) are high-affinity receptors [1]. Although it was proposed that high-affinity FcγRs are occupied by circulating IgG *in vivo* (discussed in Ref. [2]), multiple effector roles for hFcγRI, mFcγRI and mFcγRIV have been reported using mouse models of disease and therapy [3–6].

hFcγRI has been studied by exogenous expression in hFcγRI^{tg} mice, demonstrating its role on dendritic cells in the enhancement of antigen presentation and cross-presentation [7], and on neutrophils and monocyte/macrophages in inflammation, autoimmunity and systemic anaphylaxis [8]. These studies indicate that hFcγRI can, by itself, induce clinical signs of autoimmune diseases, by triggering local inflammation (*e.g.* autoimmune rheumatoid arthritis) or phagocytosis (*e.g.* autoimmune thrombocytopenia and anaemia [9]). Additionally, hFcγRI was reported to induce allergic shock (anaphylaxis) triggered by IgG-immune complexes [8]. Finally, hFcγRI may also be a therapeutic target as it can mediate antibody-based therapies such as anti-malaria [10], anti-metastatic melanoma [8] and angiogenesis prevention [6]. The mouse counterpart of hFcγRI, mFcγRI, has been studied so far only by the effect of its absence. Compared to wild-type, mFcγRI$^{-/-}$ mice demonstrate reduced reaction severity in models of autoimmune diseases

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such as experimental haemolytic anaemia and arthritis [11,12]. In addition mFcγRI$^{-/-}$ mice are less susceptible to IgG-mediated systemic anaphylaxis, Arthus reactions [13,14], and show reduced efficacy of anti-melanoma [15–17], anti-lymphoma [18] and anti-angiogenic therapies [6]. mFcγRIV was also initially studied by its absence in mFcγRIV$^{-/-}$ mice or by using blocking anti-mFcγRIV mAbs. These studies reported reduced IgG-mediated autoimmune anaemia, thrombocytopenia, rheumatoid arthritis and experimental nephrotoxic nephritis, but also reduced anaphylaxis and less efficient subcutaneous melanoma therapy in the absence or after blockade of mFcγRIV [19,20]; some of the latter results should be taken with caution since mFcγRIV-blocking antibody 9E9 may also block mFcγRIII *in vivo* [21]. Effector functions could nevertheless be definitively attributed to mFcγRIV through the generation of mice expressing mFcγRIV without other FcγR. Indeed, using mFcγRIV^{only} mice we could demonstrate that mFcγRIV can individually induce autoimmune thrombocytopenia and rheumatoid arthritis, as well as IgG-mediated airway inflammation and anaphylaxis, but not anti-metastatic melanoma therapy [8,22–24]. Altogether these data propose multiple effector functions for high-affinity receptors hFcγRI, mFcγRI and mFcγRIV in autoimmune and inflammatory disease models, and therapy, with direct evidence provided by studies using hFcγRI^{tg} mice and mFcγRIV^{only} mice, but only indirect evidence provided by mFcγRI$^{-/-}$ mice.

The *in vivo* effector functions proposed for mFcγRI in IgG-mediated autoimmune disease and therapy models are surprising, considering its expression is restricted to monocytes, monocyte-derived dendritic cells and some tissue-resident macrophages, and is absent on neutrophils. As several reports suggest redundant functions among mFcγRs (reviewed in Refs. [1,2]), it is uncertain if mFcγRI can induce IgG-mediated autoimmune diseases and therapeutic efficacy by itself, or if this receptor is indirectly involved: either for optimal activation via other mFcγRs or priming of effector cells. Therefore we analysed the *in vivo* effector functions of mFcγRI in mFcγRI^{only} mice, *i.e.* in the absence of mFcγRIIB, mFcγRIII and mFcγRIV, in comparison with mFcγR^{null} mice that express no mFcγR. Our results identify the effector functions of mFcγRI as more restricted than initially reported, but confirm that mFcγRI does function independently *in vivo*, in particular for depletion of IgG-opsonised cells.

2. Materials & methods

2.1. Mice

C57BL/6J mice (WT) were purchased from Charles River. VG1505 (FcγRI^{only}) mice were reported previously [17] and generated by Regeneron Pharmaceuticals, Inc. FcγR^{null} mice were generated by crossing FcγRI^{only} mice to FcγRI$^{-/-}$ mice. FcγRI^{only} and FcγR^{null} mice were bred at Institut Pasteur, used for experiments at 8–11 weeks of age. Experiments using mice were validated by the CETEA ethics committee number 89 (Institut Pasteur, Paris, France) under #2013-0103, and by the French Ministry of Research under agreement #00513.02.

2.2. K/BxN serum-induced passive arthritis

K/BxN serum was generated from a pooled collection of >40 animals. Arthritis was induced by i.v. transfer of indicated volumes of K/BxN serum, and scored as described [25]. In some experiments mice were sacrificed on day 8 for blinded histological assessment.

2.3. Airway inflammation

As previously described [26], mice were injected with 50 μL of rabbit anti-OVA serum i.n. and 500 μg of OVA i.v. 16–18 h later 4 bronchoalveolar lavages (BALs) were performed with cold PBS (1 × 0.5 ml, then 3 × 1 mL) under lethal anaesthetic. Cells were pooled and stained for flow cytometry after RBC lysis; and haemorrhage was determined by OD570 nm in the supernatant. We confirmed that mFcγRI, like all mouse FcγR, can indeed bind rabbit IgG immune complexes (Supplementary Fig. 2).

2.4. Passive systemic anaphylaxis (PSA)

Mice were sensitised by i.v. injection of 500 μg anti-DNP IgG2a (clone Hy1.2) and challenged 16 h later with 200 μg TNP(21-31)-BSA i.v. Alternatively, mice were injected with 1 mg of heat-aggregated (1 h at 63 °C in BBS pH8) human IVIG; considering that mouse FcγRI cross-binds human IgG subclasses 1, 3 and 4 [27]. Central temperature was monitored using a digital thermometer with rectal probe (YSI).

2.5. Experimental thrombocytopenia

Blood samples were taken in EDTA before and at indicated time points after i.v. injection of 3 or 10 μg anti-platelet mAb 6A6 (mouse IgG2a). Some mice were treated 32 h before 6A6 injection with 300 μL PBS- or clodronate-liposomes i.v. Platelet counts were determined using an ABC Vet automatic blood analyser (Horiba ABX).

2.6. Tumour immunotherapy

Mice were depilated and received 5 × 10^4 B16-Luc2+ cells s.c. on d0. Where indicated, mice were injected i.v. with 200 μg mAb TA99 on d1, d2 and d3 (Fig. 4A, closed symbols), and control groups were untreated (Fig. 4A, open symbols). Bioluminescence was acquired from anaesthetised mice on d1, d7 and d13, 10 min after injection of 75 μg luciferin s.c. (IVIS Spectrum CT, Caliper Life Sciences), and images were analysed with Living Image software.

2.7. Anti-mouse CD20 treatment

Mice received a single i.v. injection of 50 μg anti-mouse CD20 (clone 5D2, IgG2a, Genentech) to deplete endogenous B cells, or saline control, and $CD19^+B220^+$ B cells in the blood, spleen and inguinal lymph nodes were assessed 16 h later by flow cytometry. Remaining B cells were calculated as a percentage of the average of vehicle-treated controls (Fig. 4B).

2.8. Statistics

Data was analysed using one-way ANOVA with Bonferroni post-test (Fig. 2C) or a Tukey's multiple comparisons test (Fig. 1B) to compare individual timepoints (Fig. 2 B, D & E, and Fig. 3 A, B & E), or a Kruskal-Wallis test with Dunn's multiple comparisons (Fig. 2A and C, bottom panel); a Student's t-*test* (Fig. 3 C&D, Fig. 4B) or a Mann-Whitney test (Fig. 4A). Statistical significance is indicated (ns: $p > 0.05$; *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

Please refer to Supplemental methods for information on reagents, flow cytometry, histology, surgical procedures and Active Systemic Anaphylaxis (ASA).

3. Results & discussion

To evaluate the *in vivo* effector functions of mouse FcγRI, we investigated mice expressing this receptor in the absence of other endogenous classical FcγR (FcγRIIB, FcγRIII and FcγRIV-deficient), termed FcγRI^{only} mice [17] or VG1505 mice [28], in comparison