Inhibitory IgG Receptor-Expressing Cells: The Must-Have Accessory for Anti-CD40 Immunomodulatory mAb Efficacy

Pierre Bruhns, Jean-Luc Teillaud

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Pierre Bruhns[1,2] and Jean-Luc Teillaud[3]

Authors' affiliations

[1]Institut Pasteur, Department of Immunology, Unit of Antibodies in Therapy and Pathology, Paris, France;

[2]INSERM, U1222, Paris, France;

[3]Cordeliers Research Center-INSERM UMR-S 1138, Paris, France;

ABSTRACT

In this issue of Cancer Cell, Dahan and colleagues demonstrate that modified Fc regions of immunomodulatory anti-human CD40 mAbs can drastically increase their efficiency in a pre-clinical model expressing human CD40 and human IgG receptors. This study also highlights the fine balance between increased treatment efficacy and unwanted secondary effects.

TEXT

Monoclonal antibody (mAb) strategies to fight cancer have branched into two major routes: one aimed at targeting directly tumor antigens to reduce tumor growth or induce tumor killing (*i.e.* anti-tumor mAbs); one aimed at enhancing/unlocking the activation of specific immune cells to induce tumor killing (*i.e.* anti-checkpoint inhibitor mAbs). Surprisingly, even though the respective mechanisms of action of these two types of mAbs are widely different and described as cell-autonomous *in vitro*, *in vivo* results using pre-clinical models have demonstrated the requirement of accessory cells for efficacy *in vivo* that share one common feature: expression of IgG Fc receptors (FcγR). In both scenarios, the therapeutic IgG mAb is required to link the target molecule -bound by its Fab portion- on the target cell to an IgG receptor -bound by its Fc portion- on a different cell type than the target cell. This mechanism was expected for most anti-tumor mAbs that indeed link tumor cells to phagocytic or cytotoxic FcγR-expressing cells (macrophages, NK cells, neutrophils). The requirement for an accessory cell type for anti-checkpoint inhibitor mAbs was, however, unexpected.

Immune checkpoints come in two flavors, stimulatory like CD40 (TNF-R superfamily molecule) on dendritic cells, or inhibitory like CTLA-4 and PD-1 on T cells and PD-L1 on dendritic cells. The aim of immune checkpoint therapeutic mAbs is either to agonistically engage the stimulatory molecules or to antagonistically engage the inhibitory molecules, both targeting strategies inducing dendritic cell activation leading to subsequent

T cell activation, or direct T cell activation. However, two independent studies demonstrated that anti-tumor effect of anti-mouse CD40 IgG mAbs required its binding to FcγRs (Li and Ravetch, 2011; White et al., 2011). Even more surprisingly, the mouse IgG receptor required for *in vivo* efficacy was not, as conventionally expected from studies using anti-tumor antigen mAbs, an activating FcγR but the inhibitory IgG receptor FcγRIIB. The contribution of FcγRIIB to this mechanism was independent of the phosphatase (SHIP-1) necessary for its inhibitory signaling, and it originated from another cell than the target cell, *i.e.* acting in *trans* (Li and Ravetch, 2013; White et al., 2011). Thus the proposed mechanism includes an accessory cell expressing FcγRIIB that serves in *trans* as a docking site for heightened aggregation of the immune checkpoint molecule on the dendritic cell surface: the clustering of CD40 induced by bivalent anti-CD40 mAbs does not appear to suffice for cell activation, but once Fc portions of anti-CD40 mAbs are also bound by FcγRIIB on another cell surface, clustering of CD40 molecules increases, enabling downstream CD40-induced signaling and subsequent dendritic cell activation and T cell priming.

This mechanism does not apply to mAbs targeting PD-1, PD-L1 (Dahan et al., 2015) or CTLA-4 (Simpson et al., 2013), thus mainly to mAbs targeting TNF-R superfamily molecules, *e.g.* CD40 and DR5 (Wilson et al., 2011). How exactly does FcγRIIB increase clustering of IgG-targeted molecules in *trans* on other cell surfaces still remains undefined, but may rely on the unique ability of some of its isoforms (B1 and B1' in mice; B1 in humans (Latour et al., 1996)) to interact with the actin cytoskeleton and induce receptor capping at the cell surface. That B cells, which express exclusively the pro-capping FcγRIIB1 isoform, are required to express FcγRIIB for anti-CD40 mAbs efficacy in mice (Li and Ravetch, 2013) is in favor of this hypothesis but remains to be demonstrated.

In this issue of Cancer Cell, Dahan and colleagues developed a novel and elegant pre-clinical model to study in detail the mechanism of anti-human CD40 mAbs: a multiple transgenic mouse model expressing human CD40 (but not mouse CD40) and human FcγRs: FcγRI, hFcγRIIA(H_{131} variant), hFcγRIIB, hFcγRIIIA(F_{158} variant) and hFcγRIIIB(unknown polymorphic variant). With this model, the authors could directly evaluate the therapeutic efficacy of human therapeutic mAbs, as this model expresses both the human target molecule and the human effector/accessory FcγRs. They compared a series of Fc mutated IgG1 anti-hCD40 antibodies (clone CP-870,893) using as a reference its original IgG2 format. This novel study extends the previous findings on the necessity of mouse FcγRIIB for the efficacy of anti-mouse CD40 mAbs (Li and Ravetch, 2011; White et al., 2011) to the human system: human FcγRIIB is required for the *in vivo* efficacy of anti-human CD40 mAbs. As expected from the relative affinities of human IgG subclasses for human hFcγRIIB (Bruhns et al., 2009), IgG1 anti-hCD40 was more potent *in vivo* than IgG2 anti-hCD40.

Mutations that increase the binding to hFcγRIIB had increased efficacy *in vitro* on dendritic cell-mediated T cell activation (as expected (White et al., 2013)) and *in vivo* on the reduction of tumor burden compared to non-mutated IgG1 anti-hCD40. However, only the mutations also decreasing affinity for activating hFcγRI and activating hFcγRIIA, mutant formats V9 and V11, had a dramatic increase in efficacy both *in vitro* and *in vivo*. Authors also demonstrate using hFcγRIIA^{tg} hFcγRIIB^{tg} mice or only hFcγRIIB^{tg} mice that, indeed, binding of anti-hCD40 mAbs to activating hFcγRIIA is detrimental for *in vivo* efficacy. If such a mechanism holds also true for hFcγRI^{tg} mice has not been evaluated. These results are in contrast with *in vitro* results describing activating hFcγRs expressed on cell lines providing the same help as inhibitory hFcγRIIB to anti-CD40 mAb efficacy (Wilson

et al., 2011). This discrepancy might rely on the expression pattern of hFcγRIIB *versus* activating hFcγRs *in vivo* compared to cell lines, in conjunction with the superior capping abilities of FcγRIIB isoforms. Interestingly, the same mutations that increase efficacy of anti-hCD40 mAb CP870,893 *in vivo*, enabled two other anti-CD40 mAbs (clone CD40.1 and CD40.2), inefficient in an unmutated IgG1 format, to become efficient.

Increased secondary effects may unfortunately accompany increased treatment efficacy. Patients treated with anti-hCD40 mAbs suffer indeed from transient thrombocytopenia after therapeutic mAb infusion, mainly because CD40 is expressed on human platelets. Anti-hCD40 mAbs can induce platelet aggregation by triggering hCD40 on platelets and induce phagocytosis of opsonized platelets by macrophages. Whereas IgG2 anti-hCD40 mAbs did not induce significant platelet depletion in $hCD40^{tg}$ $hFc\gamma R^{tg}$ mice, IgG1 formats drastically did, in non-mutated, V9 and V11 formats. A complex balance between hCD40 triggering, heightened by *trans* binding to hFcγRIIB but diminished by *trans* binding to hFcγRIIA, and phagocytosis by activating hFcγRs expressed on macrophages explains thrombocytopenia after therapeutic treatment. The interest in IgG1 V9 and V11 formats over the standard IgG2 format for increased anti-tumor efficacy is thus counterbalanced by their adverse thrombocytopenic events in this preclinical model. However, the expression of single polymorphic variants of $hFc\gamma RIIA^{R131}$ and $hFc\gamma RIIIA^{F158}$ in these transgenic mice, which poorly bind IgG2 compared to $hFc\gamma RIIA^{R131}$ and $hFc\gamma RIIIA^{V158}$ variants (Bruhns et al., 2009), may artificially diminish IgG2-induced thrombocytopenia in this model. Clinical evaluations of the gain in anti-tumor efficacy over unwanted thrombocytopenia for these novel anti-hCD40 IgG1 mutants will have to be benchmarked against the current IgG2 formats. Finally, IgG mutants that exclusively bind hFcγRIIB without affinity for any activating hFcγRs, in particular hFcγRIIA that cause platelet activation and aggregation (McKenzie et al., 1999), may reduce unwanted secondary effects of anti-hCD40 mAbs but remain to be identified.

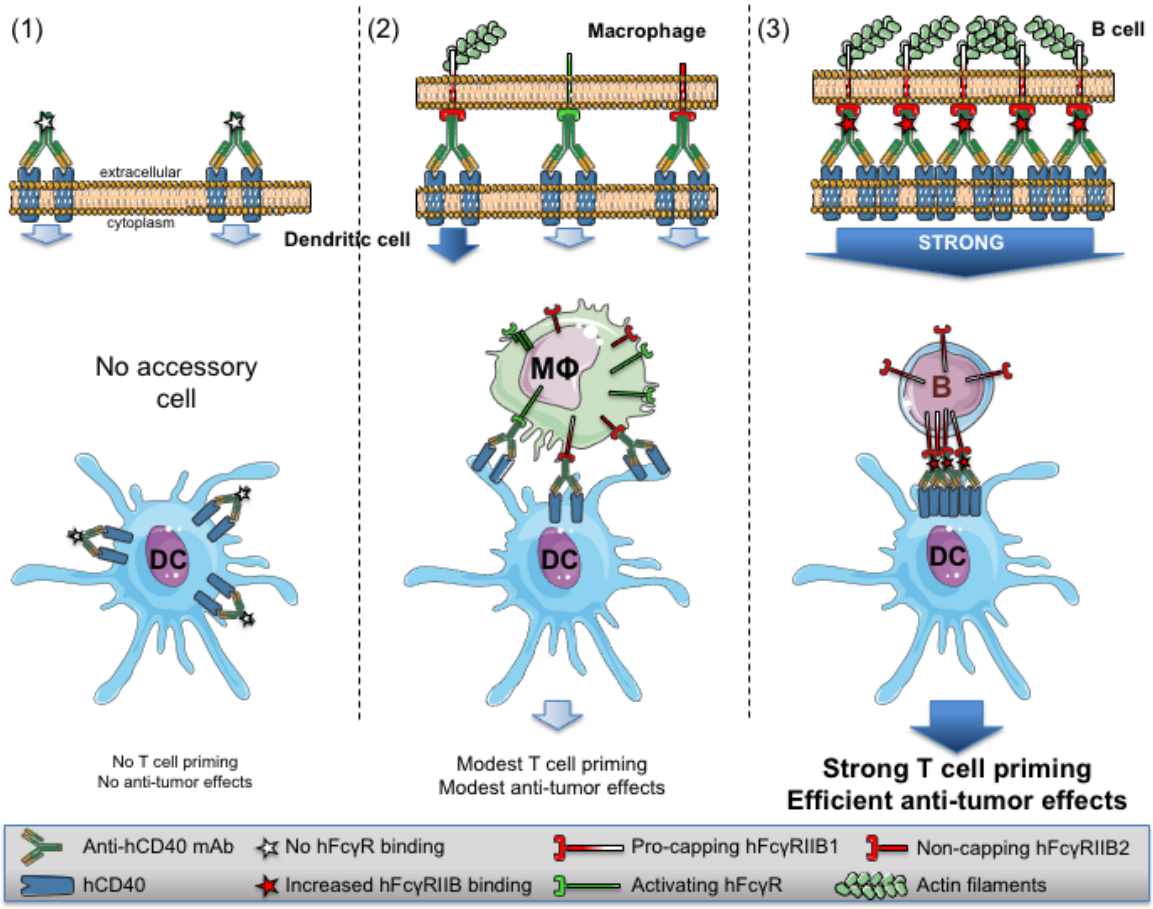

Figure 1. Mechanism of efficacy enhancement of anti-hCD40 mAbs by hFcγRIIB

Targeting of human CD40 on dendritic cells (DCs) was initially thought to require only an agonistic property for anti-hCD40 mAbs. However, recent data indicate that IgG Fc mutants of anti-hCD40 mAbs, which cannot bind hFcγRs (white star), do not generate sufficient DC activation for subsequent T cell priming and anti-tumor effects (1). Several groups proposed an "accessory" cell to be required for the efficiency of anti-CD40 mAbs, and that these "accessory" cells must express IgG receptors. If the anti-hCD40 IgG subclass binds both activating (hFcγRIIA for example) and inhibitory (hFcγRIIB) IgG receptors, "accessory" cells like macrophages may be implicated (2); the binding of anti-hCD40 in *trans* to hFcγR-expressing "accessory" macrophages modestly increases hCD40 signaling in DCs, generating modest T cell priming and anti-tumor effects. If, however, the therapeutic anti-hCD40 IgG mAb has been engineered for highly enhanced inhibitory hFcγRIIB binding (red star), "accessory" cells like B cells may predominantly be implicated, strongly increasing hCD40 signaling in DCs, T cell priming and resulting in efficient anti-tumor effects (3): the mechanism of enhancement of hCD40 signaling in DCs may rely on the cytoskeleton binding and capping ability of hFcγRIIB isoforms expressed by B cells (hFcγRIIB1), generating a DC-B cell synapse-like structure that induces hyper-aggregation of hCD40. Myeloid cells like macrophages may not generate these structures as they express both non-capping (pro-endocytic hFcγRIIB2) and pro-capping hFcγRIIB1 isoforms, as well as non-capping activating hFcγRs.

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