OncoImmunology 3, e28610; April 2014; © 2014 Landes Bioscience

Anti-human CD40 monoclonal antibody therapy is potent without FcR crosslinking

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Keywords: CD40, Fc receptor, tumor immunity, immunotherapy

Antibody agonists targeting tumor necrosis factor (TNF) superfamily receptors, including CD40, are being tested therapeutically as anticancer agents. Studies in mice have shown that anti-CD40 monoclonal antibody (mAb) requires Fc-receptor (FcR) engagement to activate antitumor immunity. In contrast, we have reported that clinically active anti-human CD40 mAb CP-870,893 does not require FcR crosslinking, a finding with translational implications.

Introduction

With the clinical value of immune checkpoint blockade convincingly established, agents that directly activate the immune system are being reconsidered. Agonist antibodies that bind tumor-necrosis factor (TNF) superfamily receptors, recognized for their ability to modulate T-cell responses, have shown efficacy against murine and human cancers and thus represent a potentially complimentary therapeutic approach to CTLA-4 or PD-1 blockade.[1,2] Monoclonal antibodies (mAb) against one such receptor, CD40, work through multiple synergistic mechanisms, activating antigen presenting cells (APC) and other cells, rather than T cells directly.[1] These mAb can also promote activity of tumoricidal macrophages.[1,3] Clinically, one agonistic anti-CD40 mAb, CP-870,893 (originally developed by Pfizer), has demonstrated activity in a spectrum of cancer patients, including those afflicted with melanoma and pancreatic adenocarcinoma.[1,3,4] Well-tolerated overall, CP-870,893 triggers transient cytokine release syndrome, manageable in the outpatient setting but a potential harbinger of its biological potency.[1]

Role of Fc Crosslinking and Epitope Specificity for CD40 Agonist mAb

It has long been appreciated that epitope fine specificity and antibody crosslinking impact the functional effects of anti-CD40 mAb in both mouse and human systems.[5,6] Recent studies of anti-mouse CD40 mAb have demonstrated that crosslinking via Fc engagement of Fc receptors (FcR) can be especially important.[2,7-10] FcγRIIB in mice has been specifically implicated as necessary for the induction of downstream signaling by anti-CD40 mAb (**Fig. 1**).[7-10] *FcγRIIB*$^{-/-}$ mice show a severely attenuated response to anti-CD40 mAb (typically rat IgG2a isotype), and the antitumor immune responses in these mice are poor.[2,7-9] Mutation of the Fc region of anti-murine CD40 to enhance FcR binding increases signal potency, suggesting that Fc engineering could be an important step for optimizing efficacy of CD40 in the clinic.[2,8,9] However, we have recently shown that the anti-human CD40 mAb CP-870,893, a fully human IgG2 molecule, does not require FcR crosslinking for potency, although CP-870,893 is the strongest agonist among the anti-CD40 mAbs currently in clinical trials.[1,2,7] Human IgG2 has a low affinity for Fc receptors and is presumably minimally crosslinked via FcR-Fc interactions in vivo.[1] We have found that antigen presenting cell (APC) activation induced by CP-870,893 does not require FcR crosslinking in vitro (**Fig. 1**), with no statistically significant difference observed in the ability of F(ab)'$_2$ CP-870,893 vs. intact CP-870,893 to stimulate B cells in culture. Artificial crosslinking by an FcγRII expressing cell line or anti-Fc antibodies did not enhance B cell activation. In contrast, an anti-mouse CD40 agonist mAb, FGK45 (rat IgG2a), does require FcR crosslinking, a necessary component both in vitro and in vivo to induce APC activation.[7] Potentially underlying these observations, we found that three crosslinking-dependent murine anti-CD40 mAbs (FGK45, 1C10, 3/23)[7-10] compete with CD40L for binding to CD40 on the surface of murine B cells, in contrast to CP-870,893, that recognizes an epitope independent of the human CD40L binding site.[7] These results suggest that the fine specificity of epitope binding plays a pivotal role in dictating the agonist potency of anti-human CD40 mAb, and further, that targeting the right epitope can bypass the need for FcR-mediated crosslinking to promote signaling through the CD40 molecule, as previously reported for mouse anti-human CD40 mAb.[5] The lower dose

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Submitted: 03/12/2014; Accepted: 03/20/2014; Published Online: 04/29/2014
Citation: Richman LP, Vonderheide RH. Anti-human CD40 monoclonal antibody therapy is potent without FcR crosslinking. OncoImmunology 2014; 3:e28610; http://dx.doi.org/10.4161/onci.28610

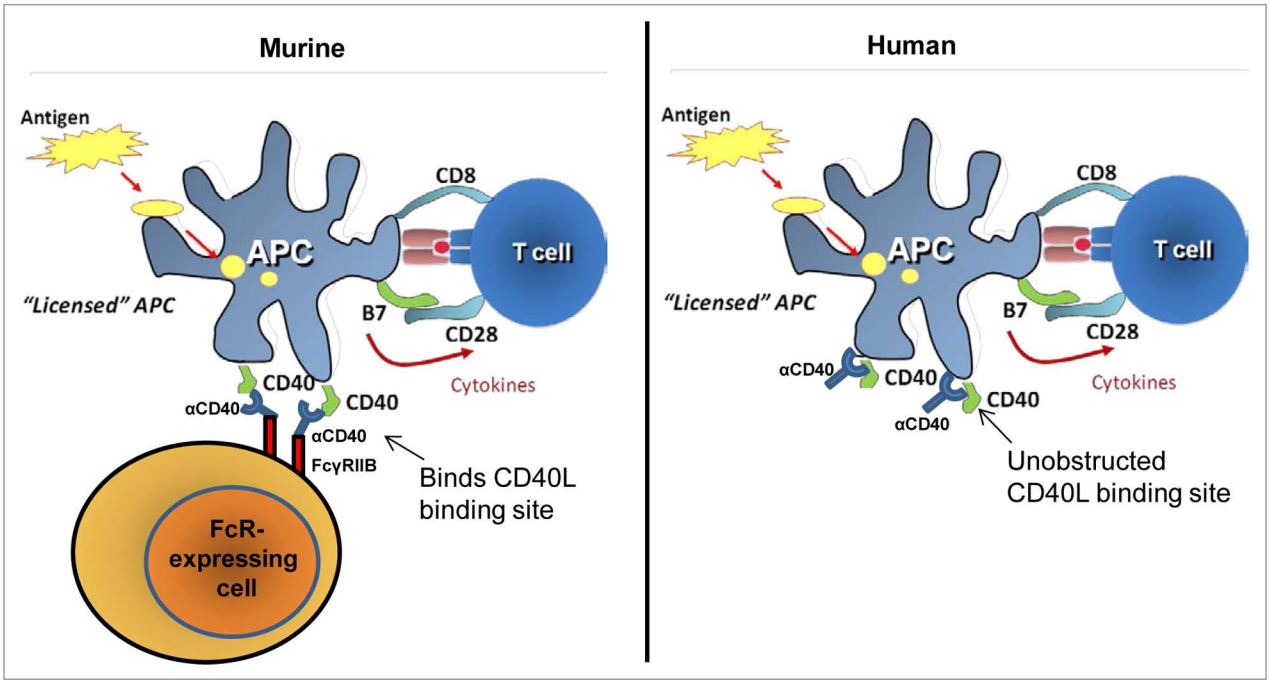

Figure 1. Differential cell-surface binding of anti-mouse vs. anti-human CD40 agonist mAb. Although each type of monoclonal antibody (mAb) can activate the CD40 pathway and license antigen presenting cells (APCs) to drive T cell immunity, anti-mouse CD40 mAb critically engage FcR crosslinking for biological activity. In contrast, FcR crosslinking is not absolutely necessary for the efficacy of anti-human CD40 mAb. This schematic is based on our studies with the anti-mouse CD40 mAb FGK45 and the anti-human IgG2 CD40 mAb CP-870,893.

of CP-870,893 needed to achieve an equivalent pharmacodynamic effect in vivo relative to that of anti-murine CD40 may reflect both epitope specificity and the nature of the Fc construct.[1,3,7]

The Next Steps for Anti-CD40 Therapy

Based on our results in vitro, it would seem unlikely that reengineering the Fc portion of CP-870,893 will necessarily improve clinical efficacy. Moreover, if conversion of CP-870,893 to IgG1 is found to increase potency in vivo, then this reagent as a therapeutic would need to be carefully reevaluated, not only for dose and toxicity but also for the potential of activation-induced immune suppression.[1] Other agonistic anti-human CD40 mAbs (mostly human IgG1) vary in potency from CP-870,893, potentially related to epitope specificity, isotype specificity, or both. Although antibody engineering is an important means to potentiate immunotherapeutic antibodies, other avenues such as alternative dosing strategies, combinations with cytotoxic chemotherapy and radiation, and combinatorial treatments of immune checkpoint-blockade mAbs represent, in our view, the most promising routes to enhance anti-CD40 clinical efficacy and drive tumor regression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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