No Need for Constant Help: Human IgG2 Antibodies Have an Autonomous Agonistic Activity for Immunotherapy of Cancer

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Agonistic antibodies specific for members of the tumor necrosis factor receptor protein family hold great promise for immunotherapy of cancer. In this issue of *Cancer Cell*, White and colleagues provide evidence that the human IgG2 subclass may represent a superior backbone for the use of these antibodies in human therapy.

Cytotoxic tumor specific antibodies have become the standard of care in patients with malignant lymphoma or breast cancer. Furthermore, the CTLA-4-specific antibody ipilimumab, which depletes regulatory T cells in the tumor microenvironment, has shown great success in cancer patients with metastatic melanoma (Simpson et al., 2013). In this issue of *Cancer Cell*, White et al. (2015) investigate how different human IgG subclasses impact the activity of a third class of antibodies holding great promise for enhancing anticancer therapies. These so-called agonistic antibodies target costimulatory molecules such as CD40, a member of the tumor necrosis factor receptor (TNFR) protein family, on dendritic cells to achieve optimal tumor-specific T cell responses, or members of the death receptor (DR) family of proteins present on tumor cells to induce apoptotic tumor cell death (Moran et al., 2013). Using these antibodies in combination with cytotoxic antibodies would allow a dual attack on tumor cells by a reduction of tumor mass via the cytotoxic antibody and an induction of strong antitumor T cell responses via the immunomodulatory antibody. While the activity of cytotoxic antibodies is critically dependent on the interaction with activating Fcγ receptors (FcγR) expressed on innate immune effector cells, one of the big surprises in the field of therapeutic antibodies was the discovery that the immunomodulatory activity of agonistic antibodies was also dependent on FcγRs and, even more unexpected, predominantly on the inhibitory FcγRIIB (Li and Ravetch, 2011; White et al., 2011; Wilson et al., 2011; Xu et al., 2003). In light of the great clinical success of the second generation cytotoxic antibodies, which have been engineered for enhanced binding to activating FcγRs, this finding provided a roadmap for optimizing the activity of agonistic antibodies by generating antibody variants with enhanced binding to the inhibitory FcγRIIB (Nimmerjahn and Ravetch, 2012). Of note, while binding of the agonistic antibody to FcγRIIB was critical, this effect did not require triggering of FcγRIIB-dependent signaling pathways, suggesting that FcγRIIB was acting as a passive cross-linker to achieve optimal induction of signaling via the target molecule bound by the agonistic antibody.

A potential issue that may arise in the clinic is that FcγRIIB may not be expressed at sufficiently high levels in all tissues, which may preclude optimal activity of at least some agonistic antibodies (Li and Ravetch, 2012). Moreover, an unwanted high level of crosslinking may result in severe side effects, such as an uncontrolled release of proinflammatory cytokines, which, according to recent data may at least in part explain the severe side effects in a clinical trial with a superagonistic CD28-specific antibody (Bartholomaeus et al., 2014). Of note, recent studies emphasize that even human IgG subclasses, such as IgG4, widely considered to have no significant binding to FcγRs can indeed interact with these receptors if the amount of antibodies bound to their target is high enough (Lux et al., 2013).

White et al. (2015) now present an elegant solution to this problem by investigating the impact of different human IgG backbones on agonistic antibody activity, demonstrating that select human IgG subclasses can trigger an agonistic activity autonomously without the need for higher-order crossliniking via FcγRs. These studies also emphasize the differences between the mouse and human antibody and the FcγR system. Whereas select IgG subclasses in mice, such as IgG1, show a strong binding to the inhibitory FcγRIIB, human IgG subclasses have a much lower affinity for their human counterpart (Figure 1). Indeed, agonistic antibodies with a human IgG1 backbone had to be engineered for enhanced binding to FcγRIIB to become therapeutically active in human FcγRIIB-transgenic mice (Li and Ravetch, 2012).

White et al. (2015) now provide convincing evidence that the human IgG2 backbone may represent a natural solution to this problem. By generating human IgG subclass switch variants specific for CD40, CD28, or 4-1BB (CD137), they show that the IgG2 subclass is superior to other IgG subclasses for induction of B cell proliferation, dendritic cell activation, and induction of tumor-specific cytotoxic T cell responses. This activity was FcγR independent in vivo, because these agonistic antibodies were fully active as F(ab)2 fragments or in mice lacking the inhibitory FcγRIIB. In a series of elegant experiments White et al. (2015) show that especially the hinge and CH1 domains of human IgG2, which are characterized by a set of distinct disulfide bonds, are critical for this effect. Thus, grafting the human IgG2 hinge and CH1-domains on an IgG1-backbone was sufficient to

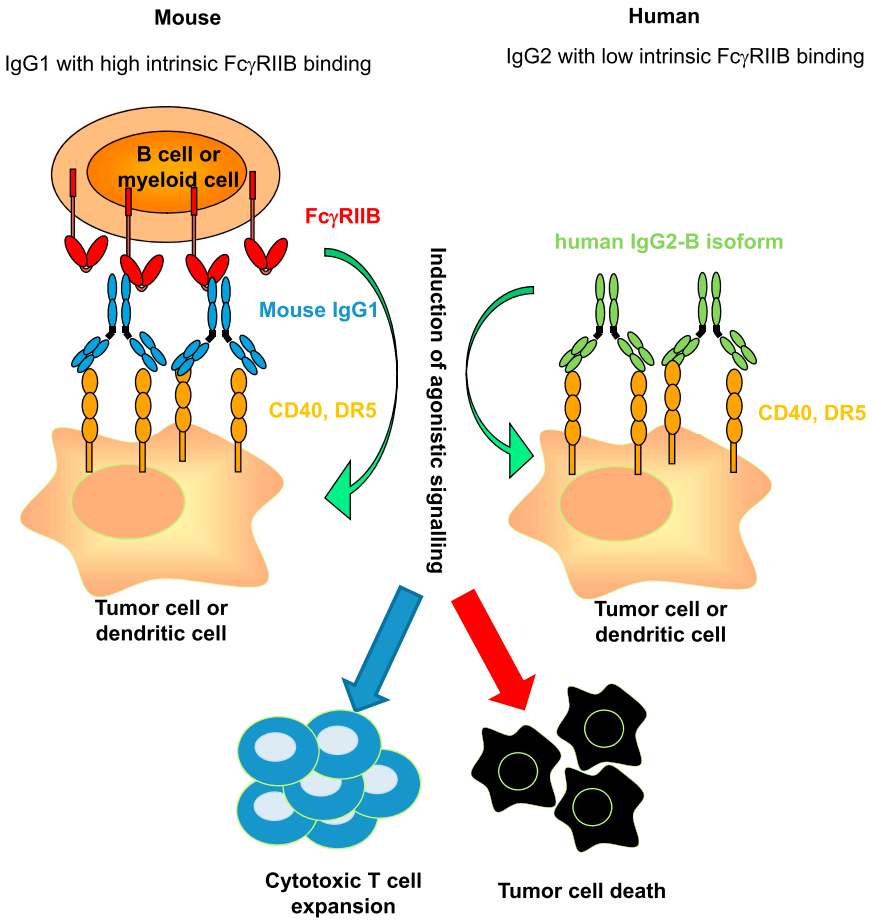

Figure 1. Pathways Responsible for the Activity of Mouse and Human Agonistic Antibody Activity In Vivo

In mice, IgG1 antibodies have sufficiently high activity to mediate agonistic effects via binding to the inhibitory FcγRIIB. In humans, the IgG2 subclass has an intrinsic capacity to trigger agonistic antibody-dependent effects and does not require higher order crosslinking. See text for further details.

transfer the agonistic activity to another antibody subclass. Going into more detail, they show that a specific subfraction of human IgG2, the so-called h2B isoform which is characterized by a more rigid structure compared to its h2A counterpart, is the major isoform responsible for this FcγR independent activity. This is of major importance, because the presence of the h2A isoform was not only inactive but was also able to block the agonistic activity of the h2B isoform. Taking these issues into account, White et al. (2015) show that protein engineering of the IgG2 backbone allows locking of the antibody in the h2B isoform, thereby obtaining optimal activity in vivo. Taken together, this study provides a clear-cut therapeutic avenue of how agonistic antibodies can be engineered with respect to safety and enhanced activity to treat human cancer.

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