Clinical Cancer Research

Immunotherapy and Hyperprogression: Unwanted Outcomes, Unclear Mechanism

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Hyperprogression (HP) is a recently defined clinical phenomenon in which patients treated with immunotherapy paradoxically exhibit rapid tumor growth. The mechanisms of hyperprogression remain ill-defined, although recent studies in this issue point to a possible role for Fc receptors in this process.

See related article by Lo Russo et al., p. 989

In this issue of *Clinical Cancer Research*, Lo Russo and colleagues describe potential cellular mediators of hyperprogression in non–small cell lung cancer (NSCLC; ref. 1). Although the clinical phenomenon of hyperprogression remains ill-defined, this and work of others highlights an important cautionary tale of how little we know about the mechanism of these therapies.

The majority of immune checkpoint inhibitors (ICI) in the clinic are recombinant IgG antibodies targeting various cell surface proteins (e.g., PD-1, CTLA-4). Antibodies are bifunctional molecules containing two identical antigen-binding domains (Fabs) and a single Fc (fragment crystallizable). While nature has endowed antibodies with incredible diversity in their Fab domains through somatic hypermutation, it has also evolved diversity within the Fc fragment (2). This is most evident by the divergent subclass activity of various immunoglobulin G proteins through altered binding to Fc receptors (FcR). In humans, there are four IgG subclasses (IgG1–4). These unique subclasses exhibit varying affinity for activating or inhibitory FcRs. There are three main activating receptors (FcγRI, FcγRIIa, and FcγRIIIa) and a sole inhibitory receptor (FcγRIIb; Fig. 1A). It is the collective balance of signaling through these receptors expressed on any given cell type that determines its effector function (e.g., antibody-dependent cellular cytotoxicity or cellular phagocytosis, ADCC/ADCP). In the context of tumors, it is important to not only consider the cells composing the immune infiltrate, but the FcRs they express and the role that different inflammatory cytokines exert on the FcR expression profile.

In general, most cytotoxic antibodies are designed using an IgG1 backbone favoring binding to activating receptors initiating ADCC/ADCP. In contrast, IgG4 antibodies have weak binding to activating receptors and are favored as agents looking to block the *in vivo* activity of a pathway. Two examples of this are antibodies blocking PD-1 and PD-L1. PD-1 antibodies are usually an IgG4 subclass in which Fc receptor engagement is not required for *in vivo* antitumor activity (3). In contrast, antibodies directed against PD-L1 are of an IgG1 subclass, thus favoring ADCC/ADCP. Preclinical studies testing the contributions of the antibody Fc for PD-L1 variants demonstrated they function, in part, through depletion of intratumoral myeloid cells. Thus, although this has not yet been definitively established in humans there are likely key differences in how ICIs work. The idea that they all fall into the same class is an extreme oversimplification. Much work has been done to define predictors of response to ICI; however, it is equally important to consider which features of a tumor could predispose to immune-related adverse events (irAE) or tumor progression.

Here, Lo Russo and colleagues evaluated a cohort of 187 patients with NSCLC being treated with ICIs (1). They defined hyperprogression as those having (i) treatment failure within 2 months, (ii) increase in target lesions >50%, (iii) significant clinical deterioration or (iv) appearance of 2 or more new lesions or new organ involvement when compared with the previous scan. Using baseline IHC, multiparameter flow cytometry, and immunodeficient mouse models, they aimed to define correlates of hyperprogression in patients receiving ICIs. They found hyperprogression at a rate of 25% in their patient population, which is on the high end of what has been previously reported (between 9% and 29%). Pathologically, they defined a population of tumor-associated macrophages (TAM) that were enriched in patients with hyperprogression. These TAMs were polarized to an M2-like $CD163^+CD33^+PD\text{-}L1^+$ phenotype in the 11 hyperprogression patients versus 24 patients without hyperprogression. Interestingly, this phenotype and clustering of TAMs was recapitulated in their xenografted tumor models. No differences were noted in infiltrating lymphocytes including CD4, CD8, or FOXP3-expressing cells. They did not appear to evaluate PD-1 expression in these samples but do note the low level (<1%) PD-1 expression in the tumor cell lines tested.

The authors then went on to see whether they could recapitulate hyperprogression in murine models as has been previously demonstrated (4). To do this, they used two separate immunodeficient models both lacking mouse T cells. Thus, the resultant $CD45^+$ infiltrating cells within the tumor microenvironment (TME) are primarily myeloid cells. They demonstrated that mice bearing H460 NSCLC tumors showed faster tumor growth when treated with the rat IgG2a anti–PD-1 antibody and was associated with an increase in tumor-infiltrating $CD45^+$ cells. This suggested a possible mechanism for PD-1–expressing myeloid cells within the TME contributing to this process. Although PD-1–expressing myeloid cells have been described, this biology remains in its infancy. Because tumor-infiltrating myeloid cells express high levels of activating and inhibitory FcRs (5), the authors went on

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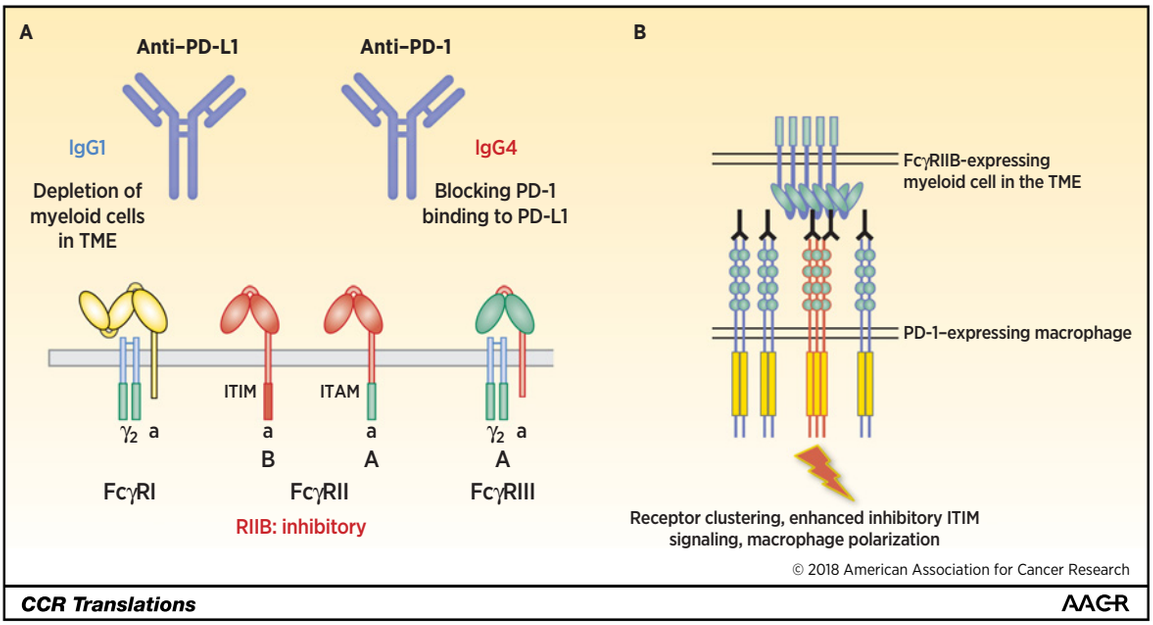

Figure 1.

A, Anti–PD-1 antibodies (IgG4) and anti–PD-L1 (IgG1) antibodies have different mechanisms of action through divergent binding to activating or inhibitory FcRs. **B,** Proposed mechanism by which FcyRIIB-enhanced clustering of PD-1 on macrophages leads to polarization through enhanced signaling through the ITIM domain. TME, tumor microenvironment.

to determine whether the mechanism of hyperprogression was Fc-receptor dependent. To do this, they generated $F(ab)_2$ fragments of the anti-mouse PD-1 antibody and saw this abrogated HP. To evaluate the role of PD-1 on infiltrating immune cells they switched to using the human version of this antibody (nivolumab) which fails to bind to mouse PD-1. In two molecularly distinct NSCLC xenograft models, they found one of them exhibited evidence of hyperprogression when treated with nivolumab and correlated with an increase in $CD11b^{+}F4/80^{high}$ myeloid cells. Collectively, the authors concluded that hyperprogression is maintained by myeloid cells within the TME and point to a potential FcR-dependent mechanism.

While these studies are intriguing and provide preliminary evidence for the cellular drivers of hyperprogression, many questions remain when determining the contribution of FcRs to this process. First, it is important to consider the clinical question in the correct experimental context. Here, an important lesson remains in that mice are not humans, where murine FcRs do not mirror the structural diversity or unique expression profile observed for human FcRs on human cells. Thus, when considering whether or not the *in vivo* activity of an antibody is Fc receptor dependent, this should be done on an FcR-deficient mouse background or with appropriately modified antibodies (e.g., mouse IgG1-D265A). While treating antibodies with pepsin removes the Fc fragment, it also severely limits any *in vivo* activity as loss of the Fc fragment alters biodistribution of drug given lack of recycling and trafficking through the neonatal Fc receptor. When $F(ab)^2$ fragments are given systemically in this model only a fraction of the product, if any, is making it to the TME. Finally, testing various ICIs in immunodeficient mice remains a severe limitation to any of these preclinical models, where immunocompetent mice carrying human FcRs in place of the mouse homologs may provide a more relevant *in vivo* system (3, 5).

In this study, treatment with anti–PD-1 antibodies in T-cell–deficient mice appears to augment tumor growth. Because this is also inhibited through depletion of host macrophages, it is possible that ICIs promote tumor growth through stimulating PD-1 on myeloid cells within the TME. Should this indeed be the case, the most likely Fc-dependent mechanism is through the inhibitory receptor, FcyRIIb. Similar to agonistic antibodies in which FcyRIIb binding is required for optimal activity *in vivo*, signaling through PD-1 in myeloid cells could be enhanced through FcyRIIb crosslinking (Fig. 1B). Prior data evaluating anti-MARCO antibodies demonstrated repolarization of TAMs was also dependent on FcyRIIb. Importantly, it has been demonstrated that Fc receptors, including FcyRIIb are upregulated in the tumor microenvironment compared with other sites (5). This could potentially serve as another biomarker identifying patients at risk for hyperprogression. Although intriguing, how do we explain the same rate of hyperprogression clinically in patients with NSCLC treated with PD-1/PD-L1 antibodies as they have major differences in FcR binding? If the rates of hyperprogression are truly the same, this likely supports less of a role for FcR-dependent progression in these patients. It would also be interesting to see the results of PD-L1 treatment in their models or in PD-1–deficient hosts.

As we move forward to more clearly define hyperprogression in lung cancer and other malignancies, the role for FcRs in determining both *in vivo* antitumor activity as well as unwanted effects (e.g., hyperprogression and irAEs) should be considered.

Disclosure of Potential Conflicts of Interest

J.V. Ravetch reports receiving commercial research grants from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other author.

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