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Activation of antigen-exposed iMC-DCs at the "right place" and "right time" promotes potent anti-tumor immunity

David M. Spencer

Department of Pathology and Immunology; Baylor College of Medicine; Houston, TX USA

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To better control the "licensing" of pro-Th1 dendritic cells (DCs), Spencer and colleagues have developed a synthetic ligand-inducible chimeric receptor, iMyD88/CD40 (iMC), incorporating synergistic Toll-like receptor (TLR) and costimulatory signaling elements, permitting DC regulation in vivo within the context of an immunological synapse. This novel technology results in potent anti-cancer activity.

Due mostly to their predominant role as antigen-presenting cells (APCs) for the induction of naïve T cells, autologous dendritic cells (DCs) have been the focus of over 200 clinical trials as cellular adjuvants for neoplastic disease. Nevertheless, objective response rates remain modest in the 10% range.[1] Since their discovery in the early 70s,[2] it has become increasingly apparent that the activation state of DCs is critical for differentiating between the pro-tolerogenic state of immature DCs and the highly immunogenic state of activated or "licensed" DCs. Likewise, a panoply of pro-immunogenic cocktails have been devised to maximize ex vivo activation of autologous DCs, including so-called monocyte-derived "maturation cocktail" (i.e., TNFα, IL1β, IL-6 and PGE_2) or newer protocols that substitute Type I interferons and other adjuvants for IL-12p70-suppressing PGE_2, leading typically to faster differentiation and higher IL-12 secretion (reviewed in ref. 3). Despite improvements, ex vivo-matured DCs have still failed to reflect significant tumor responses, possible due to premature or transient release of pro-Th1 cytokines, like IL-12, in culture concomitant with activation, prior to arrival in draining LNs in a pro-Th2 state.[4] Alternative approaches that rely on DC activation in vivo using systemic adjuvants (e.g., poly(dI-dC), CD40L) after administration of immature or partially mature DCs may circumvent this problem, but instead run the risk of immune activation of untargeted DCs or activating non-DCs with deleterious sequelae. Activated non-targeted APCs, lacking tumor antigen, potentially increase the risk of autoimmunity or diluting out the desired adaptive immune response. Therefore, the ideal vaccine would ensure that antigen expression and DC activation are coordinated and occur in a spatiotemporally regulated manner.

To circumvent the challenges of regulated activation of antigen-primed DCs, we have developed a synthetic ligand-inducible adjuvant, called "iMyD88/CD40" (or "iMC"), which combines TLR/IL1R signaling with synergistic CD40 signaling, leading to very high levels of ligand-regulated IL-12p70 secretion and potent antigen-specific, anti-tumor responses in vivo (**Fig. 1**).[5] As opposed to other methods that fully activate autologous DCs ex-vivo often 24 h or more before cryopreservation, our method permits control over the timing of DC activation, allowing LN migration and potential cognate T cell interaction to occur prior to release of high-level IL-12. To achieve this level of control, we have adapted the chemically induced dimerization (CID) method to the regulation of CD40 and TLR signaling. In CID, signaling domains, like the cytoplasmic domain of CD40 (CD40c; residues 216–277), are fused to the 12 kDa, FK506-binding protein, FKBP12.[6] A Phe to Val point mutation at residue 36 of FKBP12 further ensures high affinity (~0.1 nM) and high specificity binding to the membrane-permeable, synthetic homodimeric ligands, AP1903 or non-clinical analog, AP20187.[7] Additional sequences, like a myristoylation-targeting (Myr) sequence can be added to redirect chimeric fusion proteins to subcellular locations, like the plasma membrane. In iMC, a second membrane-proximal signaling domain derived from the "universal" TLR/IL1R adaptor, MyD88, follows the Myr domain and precedes the CD40c and tandem FKBP12v36 domains.

In order to efficiently produce iMC-expressing autologous DCs (iMC-DCs), in Narayanan et al. we used Ad5 or Ad5f35-pseudotyped adenovectors to transduce mouse bone marrow-derived

Correspondence to: David M. Spencer; Email: dspencer@bcm.edu
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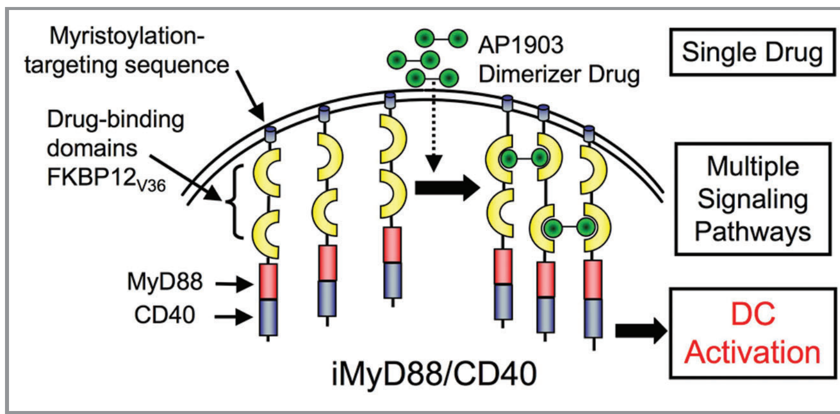

Figure 1. Schematic of iMyD88/CD40 (iMC) activation. To adapt TLR and CD40 signaling to control by membrane-permeable, synthetic dimerizer drugs (i.e., AP1903 and AP20187), The cytoplasmic domain of CD40 and a TIR domain-deleted version of MyD88 were fused adjacent to tandem copies of the modified 12 kDa FK506 binding protein ($FKBP12_{V36}$) and a myristoylation-targeting sequence. Administration of dimerizer drug in vivo leads to rapid iMC oligomerization in iMC-expressing DCs, resulting in induction of synergistic pro-inflammatory signaling pathways and ultimately DC activation.

DCs (BMDCs) and human monocyte-derived DCs (MoDCs), respectively. Following addition of AP1903 to both murine and human iMC-DCs, we observed the strong phosphorylation and hence induction of multiple signaling molecules known to be involved in TLR and CD40 signaling, including ERK, JNK, p38, Akt, IKK α/β and several NFκB subunits (i.e., p65/RelA, RelB, c-Rel). Moreover, induction of these pro-inflammatory signaling pathways correlated with elaboration of both IL-12p70 and other pro-inflammatory cytokines. We also observed high-level induction of maturation markers, such as CD40, CD86, MHC class II and CCR7, on AP1903-treated iMC-DCs, supporting the contention that CID-treated DCs were highly activated. Upregulation of CCR7 also correlated with increased migratory ability in vitro and in vivo.

Finally, we demonstrated that tumor antigen and dimerizer ligand-exposed iMC-DCs had significantly better immunogenicity against aggressive pre-established B16 tumors relative to CD40L/LPS-stimulated DCs. Moreover, iMC-DCs were also more immunogenic than DCs activated with CID-inducible CD40 (iCD40) plus LPS (i.e., iCD40-DCs). Our previous studies based on iCD40-DCs were the first to investigate the improved immunogenicity of tumor antigen-exposed DCs that could be targeted for activation following migration to lymph nodes without reliance on systemic adjuvants.[8] A phase I/IIa clinical trial, sponsored by Bellicum Pharmaceuticals, based on the use of prostate specific membrane antigen (PSMA) pulsed, iCD40-DCs (transiently exposed to LPS) in metastatic, castration-resistant prostate cancer (mCRPC) patients (BPX-101) is close to conclusion and reveals that iCD40-DCs appear safe and can trigger objective anti-tumor responses in these late stage cancer patients.[9] Use of chimeric iMC-DCs promises to be both simpler (obviating the need for transient TLR ligand) and more efficacious, driving the design on Next Generation vaccines.

In addition to efficacy, a number of factors will determine the practicality of deploying a broadly applicable DC-based vaccine. These include portability and scalability. By converting DC vaccines to a regulatable genetic element that can be co-expressed with tumor antigen(s), iMC technology moves DC vaccines closer to a viral or non-viral "off-the-shelf" weapon against cancer and potentially other pathogenic conditions. Although DC targeting in vivo is a desirable accoutrement to this technology, the high-level, pre-existing efficiency by which DCs take up small particles from their environment portends that this new drug may already be at hand.

Disclosure of Potential Conflicts of Interest

The author is a consulting officer of Bellicum Pharmaceuticals, Inc.

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