Deploying myeloid cells against myeloma

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ABSTRACT
Myeloma remains incurable despite recent therapeutic advances. We propose that minimal residual disease following cytoreductive therapy may be controlled through re-education of myeloid cells to elicit tumoricidal activity. We review work from our laboratory and others highlighting aspects of macrophage-myeloma cell crosstalk as well as strategies for therapeutic macrophage reprogramming.

Tumor-promoting macrophages in the myeloma microenvironment
Multiple myeloma, a tumor of plasma cells, is the second most common blood malignancy in the United States. Its pre-malignant form, monoclonal gammopathy of undetermined significance (MGUS) constitutes the commonest hematological condition in adults over 40. Despite therapeutic advances that have transformed treatment landscape for patients with symptomatic myeloma, two significant obstacles remain. First, the disease remains incurable for the vast majority of patients. Second, effective and non-toxic strategies to delay progression of high-risk MGUS into symptomatic myeloma remain uncertain.

Myeloma is an inflammation-driven cancer because (a) polymorphisms in cytokine and immune balance genes influence myeloma development, (b) because of the cell-autonomous and non-autonomous activation of both canonical and non-canonical NFκB in myeloma tumor cells and (c) because primary activation of certain inflammatory cells (e.g., macrophages in hereditary Gaucher’s disease) is associated with higher risk of myeloma. The cytokine milieu in inflammation-driven cancers is characterized by high IL-1β, IL-6, IL-10 levels and low IL-12 levels. Tumor-associated macrophages (e.g., colonic lamina propria macrophages in colon cancer, hepatic Kupffer cells in liver cancer) are essential in shaping this milieu that is supportive to tumor cells through activation of the NFκB and JAK/STAT pathways and suppression of helper type-1 immune responses.

We have previously reported that myeloma-associated monocytes/macrophages (MAM) actively synthesize IL-6, IL-10 and IL-1β. Because the myeloma microenvironment is essentially IL-4-free, we hypothesized that MAM may resemble “regulatory macrophages (M2b),” rather than the prototypical IL-4-educated M2a macrophages. M2b macrophages are M2 macrophages characterized by production of immunomodulatory cytokines IL-10, IL-1β, IL-6 and TNFα with suppression of IL-12. By contrast, “classically-activated” M1 macrophages produce high IL-12 and low IL-10 with variable amounts of IL-1β, IL-6 and TNFα. M2b macrophages can be generated through concurrent Fc receptor ligation and Toll-like receptor (TLR) stimulation. Immunoglobulin produced by malignant plasma cells may be immobilized in necrotic early lesions and efficiently bind to Fc receptors on recruited scavenger macrophages. TLR ligation may be provided by a variety of endogenous danger-associated molecular patterns (DAMPs) or even pathogen-associated molecular patterns (PAMPs), although the latter remains speculative. We recently showed that the TLR2/6 ligand matrix proteoglycan, versican, is abundant in myeloma lesions and may play a role in this process. Alternatively, macrophages may acquire a regulatory phenotype through interaction with mesenchymal stem/stromal cells (MSC).

Reprogramming tumor-promoting MAM into tumor-suppressive MAM
Reprogramming M2b tumor-promoting macrophages into tumor-suppressive M1 macrophages may result in tumor regression or eradication. Therapeutic macrophage repolarization may be achieved through manipulation of the CD40 pathway (Fig. 1). The Sondel group has shown that therapeutic macrophage activation can be achieved through two sequential signals, a “priming signal” consisting of CD40 stimulation and a second “triggering signal” that activates TLR signaling (such as CpG, a TLR9 agonist; or MPL, a TLR4 agonist). In seminal work from the Vonderheide group, agonistic αCD40 antibodies have elicited macrophage recruitment and stromal collapse in solid tumors.

CD40 immunotherapy in myeloma: Experience and challenges
In a recent report from our group, we showed that αCD40 agonistic immunotherapy reprograms innate immune cells to exert potent anti-myeloma activity ex vivo and in vivo. αCD40-induced anti-myeloma tumoricidal effects were largely independent of cytolytic NK, T or B cells, a finding that supports the active involvement of macrophages in this process.

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Moreover, we have shown that inhibition/deletion of the serine/threonine kinase, TPL2 (Cot/MAP3K8), synergized with αCD40-based immunotherapy to enhance anti-myeloma immunity.9 TPL2 is a MAP3Kinase (analogous to RAF kinases) that operates at the crossroads of NFκB and MAPK pathways and regulates innate cell activation and cytokine secretion. TPL2 is recruited to the active CD40 complex and regulates MAPK activation in response to CD40 signaling without affecting NFκB activation in most instances. Although TPL2 is activated by stimuli that activate macrophages, its physiological actions curb macrophage-mediated tumoricidal activity while promoting the production of crucial pro-tumor cytokines. Therefore, TPL2 can be viewed as an “innate immune checkpoint” and rationally targeted in combination with αCD40-based immunotherapies.9

The mechanism of action of strongly-agonistic αCD40 immunotherapy may be fundamentally different to that of weak-agonists or antagonists previously tested in myeloma with modest results (dacetuzumab, lucatumumab).8 Whereas strong CD40 agonists may principally work through immune-mediated mechanisms, weak-agonists/antagonists exert their effects primarily through antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-mediated cytotoxicity (CMC).8

**CD40 agonists in myeloma consolidation/maintenance: Promise and caveats**

Depth of responses in myeloma is prognostic of overall outcome. Consolidation/maintenance strategies to increase response depth rates appear to be beneficial, however the optimal regimens are still under active investigation. Our preclinical data support the testing of strongly agonistic αCD40 immunotherapy (e.g., strongly-agonistic αCD40 antibody CP-870,893) in this setting. Early clinical experience suggests an acceptable toxicity profile for CP-870,893.8 Moreover, the combination of CD40 agonistic immunotherapy with T-cell checkpoint inhibitor antibodies would make mechanistic sense and may result in significantly enhanced responses.

A potential caveat relates to the expression of CD40 by myeloma cells and concerns regarding potential stimulation of the malignant clone. Although further preclinical testing is warranted to investigate the relevant mechanisms, a few remarks can be made. First, in prior work from the Sondel group, a modest direct tumor-protective effect on CD40-expressing chronic lymphocytic leukemia (CLL) cells was overcome by the anti-tumor actions of αCD40 mediated through tumoricidal macrophages.7 Second, the available experience with CP-870,893 has demonstrated depletion effects on B cells, ostensibly through activation-induced cell death.8 Indeed, direct anti-tumor effects of CP-870,893 were seen against human B-cell tumor xenografts transplanted into immunodeficient mice.10 Assuming these observations are pertinent to malignant plasma cells, this phenomenon could generate additive or even synergistic antitumor efficacy, the latter through release of tumor antigens and improved presentation by CD40-stimulated professional antigen-presenting cells. Third, any potential growth-promoting effects of CD40 stimulation could be mitigated by TPL2 or MEK inhibition because TPL2 controls MAPK pathway activation downstream of CD40 signaling through MEK.9 Thus, inhibition of TPL2 signaling, acting both as an “innate immune checkpoint” in the microenvironment and as a modulator of CD40 pathway-mediated signals in tumor cells, may augment the benefits of αCD40-therapy.

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