A New Mechanism for Blocking Myeloid-Derived Suppressor Cells by CpG

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In this issue of Clinical Cancer Research, Zoglmeier and colleagues demonstrate that CpG via the induction of interferon-α matures myeloid-derived suppressor cells to abrogate immune suppression in two murine solid tumor models.
It is now recognized that the immune system is capable of recognizing and eliminating cancer cells but that tumors evade and suppress host immune responses to persist and spread. Immunotherapy seeks to overcome tumor-mediated immune dysfunction and activate a cell-mediated immune response against cancer cells. Such an approach holds great promise for reducing damage to collateral tissue by taking advantage of the inherent specificity of the human immune system. Systemic trafficking and monitoring by immune cells also provides for superior treatment of metastatic and inoperable lesions compared with external beam irradiation and surgical therapies. Perhaps most importantly, the generation of immunologic memory following a robust anti-tumor immune response prevents the recurrence of tumors.

In a previous issue of *Clinical Cancer Research*, Sadun *et al.* (1) showed that different tumors may elicit tolerance and immune dysfunction via distinct mechanisms, but that a common result is the lack of activated cell-mediated anti-tumor immunity. Viewed in this light, a general approach to developing effective immunotherapy for cancer would require two steps: targeted, antigen specific immune activation and concurrent reversal of tumor-driven immune suppression. Immune activation may be achieved through a variety of approaches such as tumor lysate or dendritic cell (DC) vaccination, adoptive transfer of activated T cells, and infusion of stimulatory immunoligands and cytokines. The lack of integration of methods to limit or inhibit tumor-induced immune suppression with methods of immune activation may be the reason for the modest clinical successes achieved to date in the clinic.

Immune suppressor cells are now recognized as a key component of tumor immune tolerance and a major impediment to successful immunotherapy. One suppressor population in particular, myeloid-derived suppressor cells (MDSC), has become increasingly the focus of immunotherapy studies and great strides have been made in understanding their biology. MDSC represent a heterogeneous population of immature myeloid cells that consists of myeloid progenitors of dendritic cells, granulocytes, and macrophages and mediate potent suppression of T effector responses through a variety of mechanisms (3). MDSC accumulate in the settings of cancer, chronic infection, and severe trauma or sepsis, but are rare in healthy individuals (3). In mice, MDSC are well described and comprise a CD11b+Gr-1+ population with monocytic (Ly6G-Ly6C\text{high}) and granulocytic (Ly6G\text{high}Ly6C\text{low}) subsets (3). In humans, MDSC are identified functionally and by the expression of the common myeloid marker CD33, absence of
mature myeloid and lymphoid cell lineage markers, and expression of other markers (CD66b, CD11b, IL-4Rα, CD14 and CD15) depending upon the specific cancer type (3). These suppressor cells utilize a number of mechanisms to inhibit T effector responses, including nutrient depletion (arginine and cysteine), generation of reactive oxygen and nitrogen species, expansion of Treg cells, production of vascular endothelial growth factor, and over-expression of cyclo-oxygenase 2-derived prostaglandin E\(_2\) (3). Recently our laboratory has succeeded in generating suppressive human MDSC from normal peripheral blood mononuclear cells using a cocktail of cytokines which has facilitated the study of these rare cells in patients (4). Along with such studies, the advances made recently in understanding MDSC accumulation, activation, and function will undoubtedly lead to a better understanding of their biology and treatment.

While MDSC are widely recognized as a major mediator of tumor tolerance, the identification of effective MDSC-inhibiting therapies is less certain. Previously described MDSC-targeted therapies, summarized in Table 1, work through selective depletion of MDSC (5-fluorouracil, gemcitabine, docetaxel, sunitinib), inhibition of signaling pathways (sunitinib, GW2580, amiloride), or inhibition of suppressive mechanisms (celecoxib, sildenafil, ATRA). In a previous issue of Clinical Cancer Research, Ko et al. (5) first demonstrated a decreased MDSC accumulation in renal cell carcinoma patients by treatment with sunitinib, a tyrosine kinase inhibitor with selective action on the JAK/STAT3 signaling pathway. Subsequent research has shown STAT3 signaling to be a key mediator of suppressor cell function and a more recent study has shown tumor regression when dendritic cell vaccination is combined with sunitinib therapy (6). Another approach to suppressor cell inhibition is selective depletion using chemotherapy drugs (e.g. 5-fluorouracil and cyclophosphamide for MDSC and Treg depletion, respectively, Table 1) or antibody therapy (e.g. PC61 rat anti-mouse CD25). However, suppressor cell depletion appears to have limited effects in eliciting anti-tumor immune responses in vivo due to unwanted depletion of immune effector cells and the rapid regeneration of suppressor cells by expansion and peripheral conversion. Indeed, Ko et al. (5) found no tumor regression in renal cell carcinoma patients treated with sunitinib, despite decreased accumulation and selective depletion of MDSC.

In the case of MDSC, optimal immunotherapy is likely to result from a decrease in suppressor cell accumulation and suppressive function that coincides with MDSC maturation to
immune-promoting antigen presenting populations. In this issue of Clinical Cancer Research, Zoglmeier and colleagues (2) suggest that CpG treatment, by way of IFN-α, can achieve this optimal MDSC change in tumor bearing mice to produce tumor regression and as such, may be a potent new addition to cancer immunotherapy protocols. A number of TLR agonists have been evaluated as immune adjuvants for cancer therapy. Among these, CpG oligodeoxynucleotides (ODNs), which consist of unmethylated CpG dinucleotides arranged in a specific sequence and framework known as CpG motifs, trigger the production of T-helper 1 and pro-inflammatory cytokines and stimulate the activation of professional antigen-presenting cells (15). Unmethylated CpG ODNs behave as immune adjuvants that accelerate and enhance antigen-specific antibody responses and are now thought to play a large role in the effectiveness of Freund’s Adjuvant and Bacillus Calmette-Guerin (BCG) (16). CpG ODNs have also been used in tumor immunology in combination with antitumor antibodies to achieve tumor regression especially when injected intratumorally (17). However, some studies have identified a role for some TLR agonists in the expansion and/or activation of MDSC in tumor-bearing hosts (Figure 1) (18) and brought into question the use of these immune stimulants in immunotherapy protocols.

Zoglmeier et al. (2) describe a novel mechanism of action by which the toll-like receptor (TLR) 9 agonist CpG reduces MDSC suppression. The authors demonstrate for the first time that interferon-α induced by CpG treatment in tumor bearing mice differentiates MDSC to reduce their immunosuppressive activity thereby enabling a more vigorous anti-tumor immune response in the Colon 26 tumor model, and to a lesser extent, in CEA424-Tag mice bearing autochthonous gastric tumors. More specifically, CpG maturation of MDSC was most pronounced on the Ly6G$_{high}$ polymorphonuclear subset of MDSC which is the dominant population associated with immunosuppression in these models. The study further shows that interferon-α produced by plasmacytoid dendritic cells after CpG stimulation is the major effector mechanism for MDSC maturation and loss of suppressive function in vitro and that interferon-α treatment of tumor-bearing mice is sufficient to block MDSC suppressivity. Zoglmeier and colleagues (2) clarify the role of TLR agonists, showing that TLR agonists eliciting strong IFNα responses (e.g. TLR 9 agonist CpG and TLR 3 agonist poly I:C) can decrease suppressive functions and increase maturation of MDSC in contrast to the TLR 4 agonist lipopolysaccharide which promotes activation of MDSC suppressive functions (Figure 1). It remains unclear as to the effect of CpG
immunotherapy on the effect of other immune suppressor cell populations, namely regulatory T cells, but these results highlight a potential MDSC-targeted therapy and elucidate a novel mechanism of action for CpG immunotherapy.

Figure Legend

Figure 1. Schematic diagram describing the role of Toll-like receptors in the expansion and function of murine MDSC. Toll-like receptor agonists expand and activate MDSC precursors into functionally suppressive cells. Treatment with CpG, however, activates plasmacytoid DC to produce IFN-α which matures these cells into non-suppressive APCs.
Table 1. Summary of current therapies for MDSC.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cancer or Tumor Model</th>
<th>Therapeutic effect</th>
<th>Reference</th>
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<td><strong>All-trans retinoic acid</strong></td>
<td>CT-26 colon carcinoma, EL4 thymoma, and MC38 colon carcinoma murine tumor models; Renal cell carcinoma patients</td>
<td>Differentiation of MDSC into mature myeloid cells via neutralization of ROS by GSH</td>
<td>Nefedova et al.7</td>
</tr>
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<td>(ATRA) Vitamin A derivative</td>
<td>CT-26 colon carcinoma, EL4 thymoma, and MC38 colon carcinoma murine tumor models; Renal cell carcinoma patients</td>
<td>Inhibition of tumor-derived exosome-associated Hsp72 triggered STAT3 activation in MDSC by inhibition of exosome formation</td>
<td>Chalmin et al.8</td>
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<td><strong>Amiloride</strong></td>
<td>EL4 thymoma, CT-26 colon carcinoma, and TS/A mammary carcinoma murine tumor models; H23 lung adenocarcinoma human cancer cell line</td>
<td>Inhibition of tumor-derived exosome-associated Hsp72 triggered STAT3 activation in MDSC by inhibition of exosome formation</td>
<td>Chalmin et al.8</td>
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<td>K⁺-sparing diuretic</td>
<td>AB1 mesothelioma murine tumor model</td>
<td>Decreased accumulation of MDSC in spleens of tumor-bearing mice and decreased ROS production by granulocytic MDSC</td>
<td>Veltman et al.9</td>
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<tr>
<td><strong>Celecoxib</strong></td>
<td>CT-26 colon carcinoma and CEA242-Tag murine tumor models</td>
<td>Decreased suppressive function and maturation of Ly6G&lt;sup&gt;high&lt;/sup&gt; MDSC</td>
<td>Zoglmeier et al.1</td>
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<tr>
<td>COX2 inhibitor</td>
<td>AB1 mesothelioma murine tumor model</td>
<td>Decreased accumulation of MDSC in spleens of tumor-bearing mice and decreased ROS production by granulocytic MDSC</td>
<td>Veltman et al.9</td>
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<td><strong>CpG ODNs</strong></td>
<td>CT-26 colon carcinoma and CEA242-Tag murine tumor models</td>
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<td>Veltman et al.9</td>
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<td>Toll-like receptor 9 agonist</td>
<td>AB1 mesothelioma murine tumor model</td>
<td>Decreased accumulation of MDSC in spleens of tumor-bearing mice and decreased ROS production by granulocytic MDSC</td>
<td>Veltman et al.9</td>
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<td><strong>Docetaxel</strong></td>
<td>4T1-Neu mammary carcinoma tumor model</td>
<td>Polarization of MDSC toward a type 1 macrophage (M1) phenotype, selective depletion of type 2 (mannose receptor&lt;sup&gt;+&lt;/sup&gt;) MDSC over M1 cells, and inhibition of STAT3</td>
<td>Kodumudi et al.10</td>
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<tr>
<td>Antimicrotubule chemotherapeutic</td>
<td>4T1-Neu mammary carcinoma tumor model</td>
<td>Polarization of MDSC toward a type 1 macrophage (M1) phenotype, selective depletion of type 2 (mannose receptor&lt;sup&gt;+&lt;/sup&gt;) MDSC over M1 cells, and inhibition of STAT3</td>
<td>Kodumudi et al.10</td>
</tr>
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<td><strong>5-Fluorouracil</strong></td>
<td>EL4 thymoma murine tumor model</td>
<td>Selective cytotoxic depletion of MDSC in the tumor and secondary lymphoid organs of tumor bearing mice</td>
<td>Vincent et al.11</td>
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<td>Pyrimidine analog chemotherapeutic</td>
<td>EL4 thymoma murine tumor model</td>
<td>Selective cytotoxic depletion of MDSC in the tumor and secondary lymphoid organs of tumor bearing mice</td>
<td>Vincent et al.11</td>
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<td><strong>Gemcitabine</strong></td>
<td>4T1 mammary carcinoma murine tumor model</td>
<td>Selective cytotoxic depletion of MDSC in the tumor and secondary lymphoid organs of tumor bearing mice</td>
<td>Le et al.12</td>
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<td><strong>GW2580</strong></td>
<td>3LL lung carcinoma, RM-1 prostate carcinoma, and B16F1 melanoma tumor models</td>
<td>Inhibition of CSF1 signaling decreases recruitment of TAM and monocyte MDSC</td>
<td>Priceman et al.13</td>
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<td>Inhibitor of CSF receptor signaling</td>
<td>3LL lung carcinoma, RM-1 prostate carcinoma, and B16F1 melanoma tumor models</td>
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<td>Priceman et al.13</td>
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<td><strong>Sildenafil</strong></td>
<td>CT-26 colon carcinoma, 4T1 mammary carcinoma, and A20 B-cell lymphoma murine tumor models</td>
<td>Down regulation of MDSC suppressive marker IL-4Rα</td>
<td>Serafini et al.14</td>
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<td>Phosphodiesterase type 5 inhibitor</td>
<td>CT-26 colon carcinoma, 4T1 mammary carcinoma, and A20 B-cell lymphoma murine tumor models</td>
<td>Down regulation of MDSC suppressive marker IL-4Rα</td>
<td>Serafini et al.14</td>
</tr>
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<td><strong>Sunitinib</strong></td>
<td>Renal cell carcinoma patients</td>
<td>Decreased MDSC accumulation in cancer patients and decreased viability and suppressive function in vitro</td>
<td>Ko et al.5</td>
</tr>
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<td>Tyrosine kinase small molecule inhibitor</td>
<td>Renal cell carcinoma patients</td>
<td>Decreased MDSC accumulation in the tumor microenvironment and improved cancer vaccine efficacy</td>
<td>Bose et al.6</td>
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</tbody>
</table>
References


Expansion

Murine MDSC

CD11b+ Gr-1+

CpG (TLR9 agonist)

Single stranded RNA (TLR7 agonist)

Expansion

Murine MDSC

Murine MDSC

TLR2, 3, 4, 7 and 9 agonists

Activation

Activated murine MDSC

LPS (TLR4 agonist)

ARG-1, iNOS, NADPH oxidase (Suppressive)

Plasmacytoid DC

IFNα

Poly I:C (TLR3 agonist)

Mature APC

CD11c+, MHC II+, CD80+, Ly6C+, Sca-1+ (Non-suppressive)
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