TLR8 agonist VTX-2337 (motolimod) decreases monocytic myeloid-derived suppressor cell
by inducing cell death

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Introduction:

- Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells and myeloid progenitor cells.
- MDSCs are increased in cancer patients and inhibit anti-tumor immune responses and produce mediators that promote the growth and survival of tumor cells.
- Inhibition or depletion of MDSCs enhances the activity of cancer vaccines in animal models.
- VTX-2337, motolimod, is a selective TLR8 agonist designed to mobilize a patient’s own immune system by directly activating myeloid dendritic cells, monocytes and natural killer cells.
- In this study, we propose to examine the effect of motolimod on MDSC function.

Methods:

Cell isolation. PBMCs from normal donors were stained with fluorochrome-conjugated monoclonal antibodies, HLA-DR, CD14, CD11a, and CD123, for the isolation of monocytes (mDC, HLA-DR+CD14+), monocyte-derived DC (mDC, HLA-DR+, CD11C+), plasmacytoid DC (pDC, HLA-DR+, CD123+). The cells were stained with Lin, HLA-DR, CD14, and CD33 for isolation of monocyte MDSC (m-MDSC), HLA-DR+CD14+ and granulocytic MDSC (g-MDSC, HLA-DR-, Lin-CD33+). The cells were separated using BD FACs Aria cell sorter.

TLR gene expression analysis. Total RNA was extracted from FACs-sorted cells using a RNA Easy Mini Extraction Kit. cDNA synthesis and real-time RT-PCR were performed using primers and probes from Applied Biosystems. The levels of mRNA expression of TLR7, 8 and 9 were normalized to b-actin/HPRT

Cell Culture. MDSC populations from PBMCs of healthy donors were analyzed after motolimod treatment. The cells were cultured with medium and PBS, low dose (167 nM) or high dose (500 nM) motolimod (VTX-2337) for 8 hours. PBMCs were also cultured with medium and PBS, 10mM and 50mM of TLR7/8 agonists (Imiquimod and 50mM and 100mM of TLR9 agonist CpG) and analyzed by FACS.

Flow cytometry. After culture, cells were stained with appropriate antibodies: CD14 APC-Cy7, HLA-DR PerCP-Cy5.5, CD33 APC, and Lineage FITC. The stained cells were acquired with FACs Canto cytometer and analyzed using FlowJo software.

Apoptosis Analysis. After 8 hours of motolimod treatment, total cells were collected and stained with the PE Annex V Apoptosis detection kit. The stained cells were acquired with FACS Canto flow cytometer and analyzed using FlowJo software. Results were reported as % of dead cells.

Summary:

- TLR8 levels found in the m-MDSC subpopulation were comparable to those found in monocytes and myeloid dendritic cells (mDC), known to express high levels of TLR8 protein and activate in response to motolimod.
- Treatment with motolimod resulted in a significant loss of the m-MDSC (HLA-DR-CD14+) population.
- There was no significant decrease in g-MDSC due to motolimod treatment.
- Specificity of decrease in m-MDSCs due to TLR8, not TLR7 and TLR9.
- There is a specificity of decrease in m-MDSC viability resulting from TLR8 activation while PBMC populations are unresponsive to TLR8 activation.
- Monocytic MDSCs underwent apoptosis after motolimod treatment.
- Our finding, where TLR8-activated m-MDSCs undergo apoptosis, suggests the potential for using motolimod to modulate MDSCs in cancer patients and enable a more effective, immune response to tumors.
- This innovative approach to diminish m-MDSC populations, or obstruct their immunosuppressive functions, hold great promise for augmenting anti-tumor immunity.

References:


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