Targeting Innate Immunity as a Driver of the MDS Phenotype

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Age-Related Clonal Hematopoiesis (CHIP) is Linked to Risk of Inflammatory Co-morbidities

- Whole exome NGS on PB of 17,182 persons; median f/u 8 years
- 805 somatic mutations found in 73 genes from 746 (4.3%) individuals
- Majority involved 1 mutation: DNMT3A (n=403), TET2 (72), & ASXL1 (62)
- Median VAF was 0.09, ~18% of WBC
- Risk of myeloid malignancy was markedly increased in mutation carriers [HR 11, 95% CI 3.9-33] & higher VAF
- CHIP was associated with greater risk for inflammatory morbidities: Type 2 DM [OR 1.3, 95% CI 1.1-1.5], CAD [HR 2.0, 95% CI 1.2-3.4] or stroke [HR 2.6, 95% CI 1.4-4.8]

Inflammaging: TLR Signaling Skews HSPC Toward Myelopoiesis with Senescence

- Age-related decline in HSPC homeostasis is linked to TLR-induced inflammatory cytokines (e.g., IL-6, IL-1β, TNFα) by MSC.
- TLR ligation drives GMP expansion in the absence of myeloid GFs, while reducing lymphocyte production by CLPs contributing to immunosenescence.
- Chronic TLR activated HSC lose quiescence causing HSC depletion.

Innate Immunity
An Emerging Pathogenetic Driver in MDS

- Chronic inflammation is linked to MDS predisposition with innate immune activation implicated in hematopoietic senescence & MDS pathobiology

- MDS HSPC overexpress TLR-2, -4 & -9, with TLR4 implicated in progenitor apoptosis & cytopenias (Hoffman W, Blood 2002; Wei Y, Leukemia 2013)

- TLR4 signaling intermediates, \textit{TRAF6} & \textit{TIRAP} are up-regulated or amplified in CD34+ progenitors (Gondek LP; Starczynowski DT, Blood 2008)

- TLR signaling is constitutively active in del5q MDS d/t \textit{miR-145} & \textit{miR-146} allelic deletion with \textit{TIRAP/TRAF6} de-repression (Starczynowski DT, Nat Med 2010;16:49)

- The TLR4 adaptor kinase IRAK is overexpressed & hyperactive, driving MDS HSPC expansion (Rhyasen, Cancer Cell 2013)

- Our recent studies implicate expansion of Myeloid Derived Suppressor Cells as key innate immune effectors of ineffective hematopoiesis (Wei S, JCI 2013)

\textsuperscript{^\text{Kristinsson SY, et. al. JCO 2011;29(21):2897–2903.}}

\textit{TRAF6}: tumor necrosis factor receptor- associated factor-6; \textit{TIRAP}: Toll-interleukin-1 receptor domain-containing adaptor protein.
Myeloid-DerivedSuppressor Cells (MDSC)

Immature myeloid cells (IMC)
- Mouse MDSC: CD11b+Gr-1+ (+B220, CD31); Human: Lin-HLA-DR-CD33+

Expand with age, infection, inflammation, and neoplasia.
Induce tumor immune tolerance & T-reg cell expansion.
Elaborate multiple soluble effectors: ROS, NO, and Arginase; VEGF, TNFα, TGF-β, IFN, IL-6, IL-10; & granzyme granules
MDSC expansion and activation driven by TLR ligands (e.g., DAMP signals)

*DAMP: danger-associated molecular pattern.
MDSC are Markedly Expanded in the BM of Lower Risk MDS Patients

MDS MDSC are Genetically Distinct from the MDS Clone

- MDSC lack both cytogenetic abnormalities & gene mutations intrinsic to the MDS clone
- Absence of genetic abnormalities indicates that MDS MDSC derive from non-neoplastic HSPC & precede emergence of MDS clones

MDS-MDSC Suppress Autologous Hematopoiesis


Granzyme Mobilization

Apoptosis

CD33 (red), granzyme B (green)

BFU-E

Number of BFU-E

p<0.001

Number of Colonies

Unsorted MDSC+ MDSC-

BFU-E CFU-GM
The ITIM Signaling Receptor CD33-SIGLEC3 is Over-expressed in MDS-MDSC

MDSC CD33 Surface Density

p<0.005

*Immunoreceptor tyrosine-based inhibition motif (ITIM);
Sialic Acid-binding Ig-Type Lectin

Promotes Myeloid Differentiation & Maturation

Blocks Differentiation & Maturation
S100A9 is the Native Ligand for CD33

CD33-IgG₁ Fc Fusion

CD33 Binds S100A9

Human S100A9

- S100-Calcium binding protein A9, is also known as migration inhibitory factor-related protein 14 (MRP14) or calgranulin B
- A calcium & zinc binding protein that plays a key role in the regulation of inflammation & innate immune response
- Predominant in myeloid cells & promotes membrane assembly & activation of NADPH oxidase
- S100A9 is the principal transcriptional driver of S100A8 (MRP8) & forms homo- & hetero-dimers with S100A8 (calprotectin)
- S100A9 & calprotectin function as alarmins or danger-associated molecular pattern (DAMP) signals & ligands for TLRs
- S100A9/8 increases with inflammation, aging in parallel with MDSCs and promotes insulin resistance & atherosclerosis*.

CD33 is Indispensable for S100A9 Inflammatory Cytokine Induction

Normal donor BM-MNC’s RAGE, TLR4, CD33, or their combination were blocked prior to culturing cells with or without 1 μg of S100A9 for 48 hours followed by assessment of IL-10 gene and protein expression (qPCR – top, ELISA on the bottom).
S100A9 is Increased in Lower Risk MDS BM-MNC & BM Plasma

**BM Plasma Concentration by IPSS**

![Graph showing BM Plasma Concentration by IPSS](image)

- Normal: 0.4 ug/mL
- Low Risk: 17.0 ug/mL
- High Risk: 11.9 ug/mL

**Intracellular S100A9**

- CD34+CD38
- CD34+CD38*
- CD33+
- CD71+

![Histograms showing Intracellular S100A9](image)

- Normal vs MDS comparisons:
  - CD34+CD38: 3.3 vs 22.4
  - CD34+CD38*: 15.6 vs 39.4
  - CD33+: 40.9 vs 67.0
  - CD71+: 4.8 vs 15.5

*Statistically significant difference
S100A9-Tg Mice Develop Trilineage Cytological Dysplasia Phenocopying MDS

A. Hypercellular marrow with megakaryocytic hyperplasia

B. Dysplastic megakaryocytes with single or hypolobation & increased micromegakaryocytes (dwarf megakaryocytes)

C. Hypogranulated and hyposegmented PMNs (pseudo-Pelger-Huet changes) and nuclear budding in erythroid precursors. (All cells are partially degenerated)

D. PAS stain highlights erythroid predominance

MDSCs (LIN-HLA-DR-CD33<sup>Hi</sup>) are activated & profoundly expanded in the bone marrow of MDS patients. MDS-MDSCs are genetically distinct from the MDS clone, serve as cellular effectors of ineffective hematopoiesis via direct cytotoxicity to autologous progenitors, and suppress T-cell immune response. The TLR4/CD33 ligand S100A9 promotes both autocrine-reinforced MDSC activation, & paracrine mediated myeloid progenitor cell death. Constitutive expression of S100A9 in a transgenic mouse model is sufficient for development of MDS & T2D.
Pyroptosis: Caspase-1 Dependent Inflammatory Cell Death

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<th>Characteristic</th>
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<th>Pyroptosis</th>
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<tr>
<td>Cell lysis</td>
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<td>Cation pore activation</td>
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<td>DNA fragmentation</td>
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<td>Inflammasome assembly</td>
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<td>Caspase-1 activation</td>
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<td>Caspase-3 activation</td>
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<td>Inflammatory cytokines</td>
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NLRP Inflammasomes

- Nucleotide-binding domain & oligomerization domain (NOD)-like receptor proteins (NLRP)
- Family of cytosolic pattern recognition receptors responding to danger signals triggering inflammasome formation
- NLRP3 (NALP3 or cryopyrin) forms a multiprotein complex by associating with ASC adaptor, which recruits Pro-Caspase-1 through its CARD domain
- Caspase-1 undergoes autocatalytic processing to yield two subunits that form the active caspase cleaving pro-interleukin-1 & -18

Two-Stage Induction of NLRP3 Inflammasome


PAMP denotes Pathogen-Associated Molecular Pattern; DAMP, Damage-associated Molecular Pattern or alarmin.

PAMP, DAMPs

TLR4 and IL-1R

DAMP, cation flux, ROS, cathepsin-B

NLRP, pro-Caspase-1

pro-IL-1β, pro-IL-18

Pyroptosis

Pore formation

Inflammatory molecules

DAMPs

P2X7, PANX1, TRPM2

Pore activation

NFκB

p50, p65

MyD88

IRAK1, IRAK4

TRAF6

priming

Assembly & Activation

Inflammatory stimuli

ASC

Caspase-1

p65

NLRP3

BCL-2, BCL-XL

TRPM2

P2X7

PANX1

IL-1β

IL-18

Pyroptosis Summary

- DAMP signals and oncogene mutations in MDS license a common redox-sensitive inflammasome platform to induce caspase-1-dependent pyroptotic cell death, inflammatory cytokine generation & β-catenin activation via NADPH-oxidase (NOX).
- NOX generated superoxide activates cation channels causing Ca^{++} influx and cell volume expansion.
- Neutralization of S100A9 in BM plasma or inhibition of the NLRP3 inflammasome suppresses pyroptosis, MDSC, ROS generation & nuclear β-catenin while restoring effective hematopoiesis.
- Strategies that neutralize S100A9, or inhibit inflammasome activation offer therapeutic potential in MDS.
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