Innate Immune Signaling in the Myelodysplastic Syndromes

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Synopsis

Myelodysplastic syndromes (MDS) are heterogeneous clonal hematological malignancies characterized by cytopenias due to ineffective hematopoiesis, and propensity to progress to acute myeloid leukemia. Innate immunity provides immediate protection against pathogens by coordinating activation of signaling pathways in immune cells. Given the prominent role of the innate immune pathway in regulating hematopoiesis, it is not surprising that aberrant signaling of this pathway is associated with hematological malignancies. Increased activation of the innate immune pathway may contribute to dysregulated hematopoiesis, dysplasia, and clonal expansion in MDS.
Innate Immune Signaling

The innate immune system is an evolutionarily conserved defense mechanism against pathogens\textsuperscript{1}. Unlike the adaptive immune response, the innate immune system does not recognize every foreign pathogen, but instead recognizes related components shared by many microbes, referred to as pathogen-associated molecular patterns (PAMPS). Coordinated activation of the innate immune system is achieved by key sentinel cells and a complex sequence of events, including engagement of PAMP-responsive receptors, recruitment of phagocytic cells, release of inflammatory mediators, and activation of the complement system resulting in pathogen clearance. The toll-like receptor (TLR) family plays a major role in the initial detection and subsequent elimination of foreign pathogens. This process is achieved through activation of intracellular signaling pathways, such as NF-κB and MAPK, that initiate a coordinated set of responses.

*The innate immune signaling complex is activated by functionally-related receptors*

Activation of the innate immunity pathway is the result of the interaction between a ligand (microbial product) and its cognate receptor\textsuperscript{1}. The human TLR family comprises ten members (TLR1 to TLR10) that are involved in the recognition of microbial products, such as lipopolysaccharide (LPS), lipoteichoic acid, peptidoglycan, microbial lipoproteins, and viral nucleic acids (DNA and RNA)\textsuperscript{2}. The TLRs exhibit homology to the Interleukin-1 receptors (IL-1R) and tumor necrosis factor receptors (TNFR). IL-1Rs are a functionally related family of receptors that rely on TRAFs (TNF associated factors) for transmitting intracellular signals\textsuperscript{3}. Despite the functional similarities to TLRs, IL-1Rs are not primary sensors of infection but rather secondary sensors that respond to IL-1 from sentinel cells such as macrophages that have
been previously infected. The primary role of IL-1Rs is the production of pro-inflammatory genes. The tumor necrosis factor receptor (TNFR) members (e.g., TNFR1, LT-βR, Fas, CD40, RANK, EDAR, LMP) also functionally overlap with innate immune receptors. The main purpose of the TNFR family is to activate genes involved in inflammation and immuno-regulatory responses, cell proliferation, viral responses, and growth inhibition. Ligands responsible for activation of TNFR include, TNFα, lymphotoxin (LT), Fas ligand, CD40 ligand, RANK ligand, and EDA. Research on the IL-1R and TNFR family members has revealed mechanistic similarities and related components shared with the TLRs.

Components of the innate immune signaling system

The best-described TLR member, TLR4, is the receptor for LPS. Two pathways diverge downstream of TLR4, the MyD88 (myeloid differentiation primary response gene 88)-dependent and –independent pathways, resulting in the expression of inflammatory cytokines or interferon-inducible genes, respectively (Figure 1). The MyD88-dependent pathway mediates a rapid and acute response, while the MyD88-independent pathway is responsible for a delayed response. Upon LPS recognition, a complex is formed on the outer plasma membrane consisting of LPS, TLR4, MD2 and CD14. The LPS-TLR4 interaction is stabilized by MD2 and CD14 coreceptors which then facilitates intracellular recruitment of protein adaptors required for downstream signaling. MyD88 is a TIR (toll-interleukin 1 receptor)-containing protein adaptor that forms a complex on the corresponding intracellular TIR domain of TLR4. Along with Mal/TIRAP (TIR domain containing adaptor protein), MyD88 recruits IRAK4 (interleukin-1 receptor-associated kinase) which then recruits IRAK1, resulting in subsequent autophosphorylation and disassociation from the TLR4/MyD88 complex. The MyD88-IRAK complex then binds to
TRAFs (TNF receptor associated factors), key effectors of the innate immune signaling complex. The TRAF-containing complex forms the key signaling component, which includes two kinase subunits, IKKα and IKKβ (inhibitor of NF-κB kinase alpha and beta), and a regulatory subunit (IKKγ/NEMO). One of the main TRAF members, TRAF6, is an E3 ligase that self-activates by autoubiquitination and also forms polyubiquitin lysine-63 chains on IKKγ resulting in IKK-mediated activation of NF-κB. Activation of the IKK complex by TRAF6 relies on another complex consisting of TAK1 (TGFβ activated kinase 1) and TAB1/2 (TAK1 binding protein-1 and –2). Engagement of the TAK1/TAB1/2 complex with TRAF6 not only induces NF-κB, but can also activate specific MAPK pathways (JNK and p38). In contrast, the MyD88-independent pathway forms a TLR4 complex containing TRIF and TRAM adaptor molecules (Figure 1). As in the MyD88-dependent pathway, TRAF6 plays a key role in downstream activation, but the kinases RIP1 and TBK1 are also necessary for NF-κB and IRF3 activation.

Genes regulated by innate immune signaling

Over 1000 protein-coding genes are activated following stimulation of the innate immune system. The primary goal of the innate immune system is to generate an immune response by activating the inflammatory cascade. The best characterized genes directly regulated by the innate immune pathway include cytokines (IL-1β, IL-6, TNFα, G-CSF, GM-CSF, M-CSF), chemokines (MCP-1, IL-8), and interferons (IFN-β, IRG). More recent attention has focused on the regulation of noncoding RNAs, such as microRNAs (miRNAs), by the innate immune pathway. miRNAs are 21 to 25 nucleotide noncoding RNAs that posttranscriptionally repress specific mRNA targets through 3’-untranslated region interactions. At least five microRNAs (miRs) have been reported to increase in expression following stimulation with LPS. Four
independent groups identified, miR-146, miR-147, miR-155, miR-181, and let-7 as effectors of the TLR4 pathway\textsuperscript{12-16}. The precise role of these miRNAs in mediating the LPS-TLR4 response remains to be delineated. However, mouse experiments have revealed that miR-155 may be involved in driving myeloid expansion in vivo\textsuperscript{13}, and overexpression of miR-147 abrogates a macrophage inflammatory response as indicated by lower expression of TNF\(\alpha\) and IL-6\textsuperscript{15}. The roles of miR-146, miR-181, and let-7 have yet to be studied in a similar context so it is unclear how they contribute to functional innate immune responses. However, there is some evidence that these miRNAs are important in hematopoiesis and may be involved in differentiation and/or survival of key immune cells. Knockdown of miR-146 in human or mouse hematopoietic progenitor cells results in increased megakaryopoiesis\textsuperscript{17,18}. Ectopic expression of miR-181 in hematopoietic stem/progenitor cells leads to B-lineage differentiation\textsuperscript{19}. Some of these miRNAs potentially function as negative feedback regulators. For instance, miR-146 has been shown to target TRAF6 and IRAK1\textsuperscript{12}, and let-7 and miR-181 are predicted to target TLR4\textsuperscript{14,16}. Therefore, it is conceivable that induction of these miRNAs dampens the LPS response by binding and inhibiting multiple components of the innate immune pathway (Table 1).

\textbf{Role of innate immune signaling on normal hematopoiesis}

\textit{Hematopoietic response to acute inflammation and innate immune signaling activation}

Genetic and functional studies have revealed the consequences of innate immune signaling pathways on the distinct steps of hematopoietic differentiation following inflammation and/or pathogen infection. Extensive discussion and experimental evidence on this topic has been previously reviewed\textsuperscript{1}, so only a brief overview will be provided. Inflammation and subsequent activation of innate immune signaling results in activation and expansion of mature leukocytes,
such as monocytes, dendritic cells, macrophages, and neutrophils, but reduction of B lymphopoiesis in the bone marrow\(^\text{20}\). Similarly in mice, administration of LPS through intravenous injection results in rapid and dynamic effects on myeloid and lymphoid populations in the bone marrow and blood\(^\text{13}\). After LPS injection, expansion of granulocyte/macrophage populations and reduction in B cells and erythroid precursors are observed in the bone marrow by 72 hours\(^\text{13}\). The inflammatory-mediated imbalance in lympho- and granulopoiesis is driven by growth factors, chemokines, and/or cytokines.

*Role of innate immune signaling in hematopoietic stem and progenitor cells*

Most work has described the TLRs and the innate immune signaling pathway as critical responders to foreign pathogens in mature myeloid and lymphoid cells. Emerging evidence now suggests that TLR are also important signaling transducers in hematopoietic stem and progenitor cells. Functional TLRs and their coreceptors are expressed on multipotent hematopoietic stem cells\(^\text{21}\). By binding to TLR4, LPS drives hematopoietic stem/progenitor cells to proliferate and differentiate into mature monocytes and macrophages at the expense of lymphoid differentiation in vitro\(^\text{21}\) (Figure 2a). Similarly, mice administered LPS show evidence of activated hematopoietic stem cells in the bone marrow and preferential stimulation of macrophage and monocyte development, while B and T lymphoid production is impaired\(^\text{13,20}\). These observations demonstrate a critical role of innate immune signaling in normal hematopoietic stem and progenitor cell differentiation.

The importance of TLR4 signaling in hematopoietic stem cells is further supported by genetic experiments with TAK1, a member of the MAPKKK family and a mediator of TRAF6 signaling\(^\text{22}\) (Figure 1). Under normal physiological conditions, TAK1 is expressed and activated
in hematopoietic stem cells\textsuperscript{22}. TAK1 gene-targeted mice show ineffective hematopoiesis leading to pancytopenia. Notably, TAK1-deficient mice display reduced white blood cells, platelets, and nucleated cells in the bone marrow, but hemoglobin levels are unaffected\textsuperscript{22}. Detailed examination of the marrow indicates that depletion of TAK1 results in loss of primitive hematopoietic cells in the marrow. The loss of hematopoietic stem and progenitor cells in TAK1-deficient mice is secondary to increased apoptotic signaling, mediated through loss of NF-κB and JNK activation\textsuperscript{22}. Therefore, TAK1 is a key gene of the innate immune pathway responsible for maintenance of hematopoietic stem cells.

**Role of innate immune signaling in malignant hematopoiesis**

*Innate immune signaling defect in acute myeloid leukemia*

AML results from genetic defects in a hematopoietic stem/progenitor cell or myeloid lineage precursor\textsuperscript{23}. A combination of increased cell proliferation and a block in hematopoietic differentiation are hallmark criteria for the development of AML. Classification of AML assumes morphological similarities of the leukemic cells to the normal cell counterpart within a particular stage of myeloid differentiation\textsuperscript{24}. Distinct recurring mutations have been identified in AML and are thought to be responsible for the defects associated with proliferation or differentiation of leukemic cells. Balanced translocations such as t(15;17)/PML-RARA, t(8;21)/AML1-ETO, t(16;16)/CBFB-MY11, t(9;11)/MLLT3-MLL, or other rearrangements of 11q23/MLL are common in de novo AML\textsuperscript{25,26}. These alterations, which are now part of the World Health Organization’s (WHO) classification of myeloid neoplasias, have been extensively characterized to reveal the roles of the fusion genes in leukemogenesis\textsuperscript{25,26}. In addition to genomic alterations, point mutations have also been identified. The most common mutations in AML are ones in the FMS-like tyrosine kinase 3 (FLT3) gene, CEBPA, and NPM1\textsuperscript{26}. Internal
tandem repeats of FLT3 and overexpression of MN1 are also prognostically important in AML and have been shown to directly contribute to leukemogenesis in mouse models\textsuperscript{27-30}. Identification of these alterations in AML patients has improved diagnosis and treatment, however the heterogeneity of AML and variable survival outcomes suggests that yet undiscovered genes and pathways contribute to AML.

The role of innate immune signaling in AML is becoming increasingly apparent, but the evidence is indirect. Interleukin-1\(\beta\) (IL-1\(\beta\)) has been shown to maintain survival and proliferation of AML blasts\textsuperscript{31}. Protein levels of TRAF family members are also increased in AML cell lines, but are low or undetectable in normal hematopoietic cells\textsuperscript{32}. Consistent with this observation, the downstream pathways regulated by TRAFs, such as NF-\(\kappa\)B, are activated in AML blasts but not in normal CD34\(^+\) cells\textsuperscript{33}. The importance of NF-\(\kappa\)B activation in AML is exemplified by studies using genetic and pharmacological inhibitors of NF-\(\kappa\)B. For example, inhibiting NF-\(\kappa\)B signaling suppresses growth of AML blasts and leukemic stem cells\textsuperscript{33}.

The contribution of TNFR family members to hematological malignancies is variable, and dependent on the specific receptor. TNFR superfamily members that directly associate with FADD (Fas-associated death domain) activate the extrinsic apoptotic pathway and have been reported to suppress growth of leukemic cells\textsuperscript{34}. In contrast, TNFR members that associate with TRADD (TNFR1-associated death domain) activate prosurvival signaling in parallel with apoptotic pathways, and facilitate leukemic cell growth\textsuperscript{34}. One TNFR-family member, CD30 variant (CD30v), has been identified in a significant proportion (~70\%) of AML-M4 and M5\textsuperscript{35}. CD30v is thought to induce cell growth and differentiation through interactions with TRAF2 or TRAF5 proteins and subsequent activation of NF-\(\kappa\)B in AML cell lines\textsuperscript{35}. The role of CD40 in AML is more confusing. CD40, which also belongs to the TNFR family, requires TRAFs to
propagate signals to NF-κB, STATs, and MAPK\textsuperscript{36}. Despite the unresolved role of CD40, it is clear that the ligand for CD40 (CD40L) promotes proliferation, self-renewal and increased survival potential of AML blasts\textsuperscript{37}. Furthermore, circulating levels of CD40 are elevated in AML patients and are associated with poor overall survival\textsuperscript{38}.

Individual components of the innate immune pathway have not been as extensively described in AML. Most of the reported research has described components of the MyD88-independent pathway. ASK1, a MAPK member of the MyD88-independent signal, is activated by TRAF6 in response to reactive oxidative species\textsuperscript{39}. Knockdown of ASK1 sensitizes AML cells to arsenic trioxide-mediated apoptosis\textsuperscript{40}. In contrast, azinomycin epozide induces apoptosis in AML cells through activation of ASK1 and caspase 3\textsuperscript{41}. The later observation supports the original finding that ASK1 induces apoptosis by activating p38\textsuperscript{42}. The conflicting data on the role of ASK1 in AML may be resolved by examining ASK1 in a cell-context manner. For example, ASK1 may promote prosurvival pathways in primitive hematopoietic cells, but cell death in committed myeloid progenitors. That the MyD88-independent pathway is responsible for inducing apoptotic pathways is supported by a similar pro-apoptotic role of RIP1 in myeloid blast cell differentiation\textsuperscript{43}.

More recent evidence implicating a key role for innate immune signaling in AML was provided in a miRNA expression study on cytogenetically normal AML patient samples\textsuperscript{44}. Of more than 300 miRNAs examined, only 12 correlated with overall survival. Five of these miRNAs belong to the miR-181 family. Increased expression of miR-181 family members was associated with decreased risk of death due to AML\textsuperscript{44}. Furthermore, as miRNAs are negative regulators of specific mRNA targets, this study investigated genes and proteins that were inversely correlated with miR-181 expression in the patient samples. It was revealed that genes
encoding TLRs (TLR2, TLR4, and TLR8), IL-1β, and various effectors of these pathways (e.g., CARD8, NOD2) were increased in patients with low miR-181 expression. Of these genes, TLR4, IL-1β and CARD8 are predicted targets of miR-181. Therefore, it is hypothesized that reduced levels of miR-181 results in increased signaling of the TLR4 and/or IL1R pathways and contribute to AML progression. In related studies, miR-181 family members have been associated with morphological subclasses of AML and differentiation of hematopoietic precursors.

Sequential acquisition of genomic alterations, somatic mutations, and deregulation of key signaling pathways contribute to the transformation of a normal hematopoietic stem/progenitor cell to an AML blast. The critical events result in a block in differentiation and increased proliferation of myeloid precursors. According to what is known about signaling pathways downstream of TLRs, deregulation of innate immune signaling likely contributes to resistance to pro-apoptotic signals and increased survival of AML blasts. At this time, there is no evidence to suggest that innate immune signaling mediates a block in differentiation or promotes self-renewal of primitive hematopoietic stem/precursors resulting in AML.

Innate immune signaling defects in myelodysplastic syndromes

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematologic malignancies characterized by peripheral cytopenias, a hypercellular marrow with ineffective hematopoiesis, and a propensity to progress to AML. MDS is thought to arise from a primitive hematopoietic progenitor that has acquired genetic and/or epigenetic abnormalities. The current WHO classification of MDS comprises eight subtypes, based on biological, genetic, and morphological features. Activation of the innate immune system by chronic infection can
reproduce hematological abnormalities resembling MDS, including anemia, neutropenia, thrombocytopenia, and trilineage dysplasia. As examples, chronic infection with *Leishmania* or parvovirus, have been reported to induce pancytopenia and dysplastic erythroid and myeloid precursors in the marrow. Other evidence that the innate immune system plays a role in MDS comes from the use of immune modulatory drugs for treatment of MDS. The best-known immune modulatory drug, lenalidomide, has been successfully used for treatment of MDS subtypes associated with deletion of chromosome arm 5q. These observations provide indirect evidence that activation of the innate immune pathway may mediate features of MDS.

There are few described mouse models that exhibit features of MDS. As evidenced by studies in AML, identification of genetic alterations and the creation of representative mouse model systems may provide insight into the biological mechanism and potential therapeutic targets for MDS. To further our understanding of the biological basis of MDS and identify novel therapeutics, efforts to unravel the genetic networks associated with MDS need to be undertaken.

*Evidence of direct activation of innate immune pathway genes in MDS*

We recently focused on candidate genes and their contribution to 5q- syndrome, a common subtype of MDS. 5q- syndrome originates in a hematopoietic stem cell and is defined by a heterozygous interstitial deletion of chromosome arm 5q, macrocytic anemia, neutropenia, and thrombocytosis associated with abnormal megakaryocytes. Although deletion of chromosome arm 5q was first reported over 30 years ago, the gene(s) responsible for the clinical manifestation of this disease remained unknown until recently. miRNAs encoded on chromosome 5q were investigated as possible mediators of 5q- syndrome. Deletion of chromosome 5q in MDS patients results in loss of at least 2 functionally-related miRNAs, miR-
Based on miRNA target prediction algorithms and in vitro validation experiments, TIRAP was identified as a target of miR-145, and TRAF6 was confirmed to be a target of miR-146a (Tables 1 and 2). As noted above, TIRAP and TRAF6 are two proteins that lie in the MyD88-dependent pathway of innate immune signaling (Figure 1). Therefore, loss of either of these two miRNAs results in derepression of innate immune signaling. Expression of TIRAP and TRAF6 proteins is relatively low in primitive hematopoietic cells, however, reduction in miR-145 and miR-146a levels during differentiation results in increased TIRAP and TRAF6 protein, respectively. This suggests that innate immune genes, such as TIRAP and TRAF6, are tightly regulated in hematopoietic stem/progenitor cells. Murine marrow transplant models in which miR-145 and miR-146a is reduced or TRAF6 is overexpressed, results in megakaryocytic and platelet defects, and a propensity to develop acute leukemia or marrow failure, through both cell autonomous and nonautonomous mechanisms. Loss of miR-145 and miR-146a does not result in macrocytic anemia, a key clinical finding in 5q- syndrome. However, identification of the RPS14 gene in the minimally deleted region of 5q- syndrome likely explains the macrocytic anemia. Therefore, the full spectrum of clinical features in 5q- syndrome may be explained by the collective loss of miR-145, miR-146a and RPS14.

The downstream signaling pathways activated by TRAF6 following loss of miR-145 and miR-146a are not completely evaluated in bone marrow cells. However, loss of miR-145 and miR-146a results in NF-κB activation in a TRAF6- and TIRAP-dependent manner in fibroblasts. Although NF-κB is implicated in MDS, the mechanism leading to its activation in other MDS subtypes is not clear. Compared to normal CD34+ marrow cells, NF-κB activation is significantly elevated in CD34+ bone marrow cells isolated from low-risk and high-risk MDS patients, although not in chromic myelomonocytic leukemia. A similar increase in NF-κB
activity was also documented in MDS cell lines. Blocking NF-κB resulted in apoptosis of MDS and normal CD34+ cells in vitro, suggesting that NF-κB provides survival signals in these cells in part by inducing anti-apoptotic genes. The contribution of NF-κB to marrow dysplasia has not been extensively studied, although IκBα-deficient mice exhibit dysplastic neutrophils and megakaryocytes. Taken together, myeloid dysplasia and cell survival may be a feature of constitutive NF-κB signaling in MDS (Table 2).

Given that a hallmark of MDS is increased apoptosis of bone marrow precursors and terminally differentiated cells, it is possible that TLRs and TNFR, which have been implicated in inducing apoptosis in other cell types, may similarly mediate cell death and contribute to cytopenias in MDS. TLR2 and TLR4 expression was found to be increased by a TNF-dependent mechanism in CD34+ cells isolated from the marrow of MDS patients, and the level of expression correlated with the extent of apoptosis. TNFR1 was expressed at higher levels in low-risk MDS patients, while TNFR2 expression was elevated in high-risk MDS patients. TNFR1 favors cytotoxic signaling while TNFR2 favors cytoprotective signals, thus the shift from TNFR1 to TNFR2 expression may explain the increase in prosurvival signals found in the marrow of patients with higher-risk MDS. Although TLR and TNFR upregulation is reported in the marrow of MDS patients, it is not clear whether it is the MDS clone or the normal co-resident marrow cells that exhibit elevated TLR signaling and are prone to apoptosis.

Gene expression profiling experiments of have also pointed toward a role for activation of innate immune signaling in MDS. In one study, TRAF6 and IRAK1 were overexpressed by greater than 10-fold in CD34+ marrow cells from low- and high-risk MDS patients compared to normal controls. In contrast, TRAF2 is overexpressed only in low-risk MDS and AML, but not in high-risk MDS. By DNA arrays, amplification of the TIRAP locus (chromosome 11q24.2)
and the TRAF6 locus (chromosome 11p12) have been reported\textsuperscript{72,73}. However, whether amplification of the respective loci correlates with increased TIRAP or TRAF6 mRNA expression has not yet been determined. These observations support our finding that loss of miR-145 and miR-146a results in increased protein expression of TIRAP and TRAF6, respectively, in 5q- syndrome patients (Table 3).

Dysplastic cells in MDS are not exclusively part of the abnormal clone, which suggests that nonautonomous effects contribute to dysplasia. Cytokines that have been shown to be increased in MDS patients include TNF\(\alpha\), IL-1\(\beta\), IL-6, and IL-3\textsuperscript{74-76}. One of the consequences of miR-145 and miR-146 depletion or TRAF6 activation in mouse hematopoietic cells is megakaryocytic and platelet defects due to nonautonomous effects of IL-6. Circulating IL-6 protein and IL-6 transcripts are elevated in 5q- syndrome and \(\sim\)30\% of all MDS patients\textsuperscript{74,76,77}. IL-6, an NF-\(\kappa\)B target gene, is a pleotropic cytokine that stimulates megakaryocyte proliferation, colony formation, and platelet formation\textsuperscript{78}. In line with these findings, overexpression of an IL-6 transgene in mouse marrow transplantation experiments produces thrombocytosis, anemia, and transient neutropenia with progression to leukocytosis\textsuperscript{79}. We found an inverse correlation between IL-6 and miR-145 or miR-146a expression in MDS patient marrow cells, and provide direct evidence that the paracrine effect of TRAF6-induced IL6 elicits some of the features of 5q- syndrome. Interestingly, lenalidomide, the main therapy for 5q- syndrome, suppresses expression of IL-6\textsuperscript{80}.

\textit{Evidence of indirect activation of innate immune pathway genes in MDS}

Pathways associated with or converging on the innate immune pathway are also deregulated in MDS (Table 2). Missense mutations in c-cbl, that enhance its activity and may
explain the clonal dominance in MDS, are identified in approximately 50% of MDS patients with uniparental disomy at chromosome 11q\textsuperscript{81}. Contribution of activated c-cbl to MDS may be explained partly through its activation of the MyD88-dependent pathway. c-cbl is recruited along with TRAF6 to form a complex with the intracellular domains of CD40 or TRANCE\textsuperscript{82}. Although balanced gene translocations are not as common as copy number alterations in MDS, t(9;12)(q22;p12) has been reported in patients with MDS\textsuperscript{83}. This fusion involves TEL and Syk, and results in constitutive activation of the Syk tyrosine kinase linked to the PI3K, MAPK, and STAT5 pathways in hematopoietic cells\textsuperscript{84}. Whether TEL-Syk expression induces an MDS phenotype in mice has not been investigated, however, TEL-Syk does provide growth factor independent growth in a mouse hematopoietic cell line in vitro\textsuperscript{84}. Signaling from Syk has been well studied and requires components of the innate immune pathway. Specifically, IL-1β stimulation formed an IL-1R complex with Syk, TRAF6, and Src in cell lines\textsuperscript{85}. Furthermore, TRAF6 is essential for activation of Syk-mediated signaling\textsuperscript{85}. Therefore, it is reasonable to hypothesize that the TEL-Syk fusion identified in MDS patients also requires TRAF6 signaling and contributes to the disease phenotype.

Another example of a common alteration in MDS that may result in deregulation of innate immune signaling is the deletion or epigenetic silencing of suppressor of cytokine signaling (SOCS) proteins\textsuperscript{86,87}. SOCS proteins are negative regulators of intracellular signaling, best known for inhibiting JAK kinases\textsuperscript{88}. In one study, approximately 30% of MDS patients exhibited hypermethylation of the SOCS-1 locus associated with reduced mRNA expression\textsuperscript{86}. Although the consequences of SOCS deletions in other hematological malignancies has been explored, the role of SOCS hypermethylation in MDS is not known. One possible mechanism
may involve TIRAP. SOCS-1 is a negative regulator of TIRAP and blocks activation of downstream pathways by mediating ubiquitination and subsequent degradation of TIRAP89.

Clinical implications of deregulated innate immune signaling in MDS

Given that innate immune signaling appears to contribute to myeloid hematological malignancies, it will be important to determine whether deregulation of innate immune signaling is associated with adverse outcome, clinical subtypes, or therapeutic response in MDS and AML. The data would also suggest that increased expression of TRAF6 and/or TIRAP or deletion of miR-145/miR-146 in MDS patients who do not have a deletion of chromosome arm 5q will present with similar findings to those with del (5q). The role of other innate immune genes will also need to be evaluated in patients to define the impact of their deregulation on diagnosis and prognosis, and the potential for therapeutic intervention.

To our knowledge, clinically approved drugs that target innate immune system genes are not available, although proteasome inhibitors can block NF-κB activation90. Rationale to create such drugs will depend on whether experimental knockdown or inhibition of key innate immune genes will be beneficial in leukemic model systems. There is some reason to speculate that inhibiting innate immune genes in certain subtypes will be beneficial. Although the mechanism is not known, thalidomide analogs can block LPS-induced activation of NF-κB and expression of innate immune-related genes (e.g., IL-6, TNFα, and MyD88)80,91. The therapeutic efficacy of lenalidomide in MDS and other hematologic malignancies92,93, provides an impetus to design small molecular inhibitors that target innate immune genes.

Summary
Constitutive activation of the innate immune pathway in hematopoietic cells is one likely mechanism leading to abnormal hematopoiesis associated with MDS. Unlike transient activation of the innate immune pathway by acute infection, cell intrinsic defects that cause activation of the innate immune pathway prevent normal homeostasis of differentiation, apoptosis, and proliferation in hematopoietic cells (Figure 2b). Under normal conditions, microbial-mediated activation of TLR4 signaling in hematopoietic cells results in increased expression of cytokines, myeloid-lineage skewing toward neutrophils and monocytes, and eventual elimination of activated cells (Figure 2b). In contrast, a genetic defect (or chronic exposure to a pathogen) results in constitutive and dysregulated innate immune signaling. Consequently, these cells undergo abnormal hematopoietic differentiation and increased cell death of differentiated cells, through autonomous and nonautonomous effects. Concurrently, prosurvival signals mediated by innate immune pathways maintain and expand the abnormal hematopoietic stem/progenitor cells. Expansion and/or maintenance of abnormal hematopoietic stem/progenitors allows for subsequent mutations that may promote acute leukemia.
Table 1. AML- and MDS-related microRNAs target genes of the innate immune signaling pathway.

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Innate immunity mRNA target</th>
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<tbody>
<tr>
<td>miR-145</td>
<td>TIRAP</td>
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<tr>
<td>miR-146</td>
<td>TRAF6, IRAK1</td>
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<tr>
<td>miR-147</td>
<td>TLR4</td>
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<tr>
<td>Let-7a</td>
<td>TLR4</td>
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<tr>
<td>miR-181</td>
<td>TLR2/4/8, NOD2, CARD8</td>
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Table 2. Mutations that affect innate immune signaling in hematological malignancies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role in hematological malignancy</th>
<th>Potential mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-145</td>
<td>Deleted in del (5q) MDS/AML, reduced expression in AML.</td>
<td>Results in increased TIRAP expression.</td>
</tr>
<tr>
<td>miR-146a</td>
<td>Deleted in a portion of del (5q) MDS/AML, reduced expression in AML</td>
<td>Results in increased TRAF6 and IRAK1 expression.</td>
</tr>
<tr>
<td>miR-181</td>
<td>Expression suppressed in AML.</td>
<td>Correlates with increased expression of innate immunity genes.</td>
</tr>
<tr>
<td>c-cbl</td>
<td>Activating mutations in MDS/AML, UPD in MDS.</td>
<td>May result in increased TRAF6 signaling.</td>
</tr>
<tr>
<td>Tel-Syk</td>
<td>Fusion in AML.</td>
<td>May result in increased TRAF6 signaling</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>Reduced expression due to Hypomethylation in MDS and AML.</td>
<td>May result in increased TIRAP activation.</td>
</tr>
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</table>
Table 3. Innate Immune genes implicated in hematological malignancies.

<table>
<thead>
<tr>
<th>Innate immune gene</th>
<th>Role in hematological malignancy</th>
<th>Potential mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR4</td>
<td>Overexpressed in MDS</td>
<td>Expands hematopoietic stem cells, survival of abnormal clone</td>
</tr>
<tr>
<td>TNFR</td>
<td>Overexpressed in MDS</td>
<td>Prosurvival</td>
</tr>
<tr>
<td>TIRAP</td>
<td>Increased protein expression in MDS</td>
<td>Activate NF-κB, prosurvival</td>
</tr>
<tr>
<td>TRAF6</td>
<td>Increased protein and mRNA expression in MDS</td>
<td>Activate NF-κB, expand abnormal clones, prosurvival</td>
</tr>
<tr>
<td>IRAK1</td>
<td>Overexpressed in MYST3-CREBBP AML</td>
<td>Activate NF-κB</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Activated in MDS and AML</td>
<td>Prosurvival</td>
</tr>
</tbody>
</table>
**Figure Legend.**

**Figure 1. MyD88-dependent and –independent signaling pathways.** LPS is recruited to TLR4 on the outer plasma membrane by association with MD2 and CD14. MyD88 forms a complex on the corresponding intracellular domain of TLR4. Along with Mal/TIRAP, MyD88 recruits IRAK4 and IRAK1, resulting in disassociation from the TLR4/MyD88 complex. The MyD88-IRAK complex then binds to TRAFs. This TRAF-containing complex binds TAK1 and TAB1/2, resulting in the activation of the NF-κB signalosome (IKKα, IKKβ, and IKKγ). TRAF6, an E3 ligase, along with UBC13/UEV1A forms polyubiquitin lysine-63 chains on target proteins including itself. Engagement of the TAK1/TAB1/2 complex with TRAF6, not only induces NF-κB, but can under certain cellular contexts activate the MAPK pathways. In contrast, the MyD88-independent pathway forms a TLR4 complex containing TRIF and TRAM adaptor molecules. TRIF recruits TRAF6, RIP1, and ASK1 to activate MAPK. Alternatively, a TBK1 complex is formed to activate the interferon pathway (IRF). Both pathways can also mediate activation of NF-κB through the MyD88-independent signaling cascade.

**Figure 2. Model of acute and constitutive TLR4 signaling in hematopoietic stem/progenitor cells.** A. By binding to TLR4, LPS drives hematopoietic stem/progenitor cells (grey nucleus) to proliferate and differentiate into mature monocytes and macrophages (black nuclei) at the expense of lymphoid differentiation. Clearance of the pathogen restores normal homeostasis of hematopoietic stem/progenitor cells and mature monocytes and macrophages. B. Constitutive and unregulated innate immune signaling of hematopoietic stem/progenitor cells (patterned nucleus) results in abnormal hematopoietic differentiation and increased cell death of differentiated cells, through autonomous and nonautonomous effects. Prosurvival signals
mediated by innate immune pathway maintain and expand the abnormal hematopoietic stem/progenitor. Normal homeostasis of hematopoietic stem/progenitor cells and mature monocytes and macrophages is disrupted.
References


Figure 2

A

1. Steady-state differentiation
2. Balance in survival and death

3. Exposure to microbial product
4. Activation of TLR4 signaling

5. Expression of cytokines
6. Expansion of neutrophils and monocytes

7. Clearance of activated cells
8. Restored balance in differentiation and survival

B

1. Innate immune signaling in the absence of LPS
2. Abnormal differentiation

3. Dominance of abnormal progenitor

4. Sustained abnormal differentiation
5. Increased apoptosis of committed cells