When differentiation goes viral

Courtney M. Shirley and Richard F. Ambinder

In this issue of Blood, Vrzalikova et al present evidence that Epstein-Barr virus (EBV) infection leads to the down-regulation of BLIMP1α in primary germinal center (GC) B cells. BLIMP1 is a transcription factor encoded by the PRDM1 gene. It stops cell cycling and leads to terminal differentiation of B cells to plasma cells. The authors suggest that EBV inhibition of BLIMP1α expression serves to inhibit terminal differentiation of EBV–infected cells.

From the viral perspective, inhibiting the terminal differentiation of EBV–infected lymphocytes ensures the stability of the reservoir of latently infected B cells. Studies of EBV gene expression in blood and tonsils show 2 distinct forms of viral genome replication: host-driven replication commonly referred to as latency replication, and virus-driven replication commonly referred to as lytic replication. In latency or host-driven replication, cell division is preceded by replication of cellular and viral genomes (present as nuclear plasmids). The cellular DNA polymerase replicates the viral genome. Cellular and viral genomes are partitioned into daughter cells. Replication of the viral genome does not involve virion production or viral enzymes. Expression of viral gene products that might trigger an immune response is minimized. In virus-driven replication, cell division does not occur. Replication of the viral genome involves the viral DNA polymerase and other viral enzymes. Replicated genomes are packaged into virions. Whereas latency viral gene expression is readily detected in memory B cells from healthy individuals, lytic gene expression is mainly detected in plasma cells. Host-driven viral replication in memory B cells serves to increase the numbers of virus-infected B cells without expression of many viral enzymes, transcription factors, and capsid components and thus allows the virus to elude immune surveillance. By inhibiting the terminal differentiation of EBV–infected B cells to plasma cells, inhibition of BLIMP1α preserves the ability of the virus to fly under the radar. Plasma cell differentiation, on the other hand, signals an end to such passive replication. BLIMP1 and XBP1(s) lead to plasma cell differentiation and, in parallel, trip the viral regulatory switch to viral-driven lytic replication. Thus when viral gene expression is detected in plasma cells, lytic viral genes are being expressed.

Vrzalikova and colleagues suggest that BLIMP1α inhibition is the result of the EBV latency membrane protein 1 (LMP1). LMP1 is a member of the tumor necrosis factor receptor (TNFR) superfamily and modulates cellular gene expression in many ways. Many of the LMP1 effects on cellular gene expression parallel those of BLIMP1α. However, some genes are differentially affected. BLIMP1, but not LMP1, suppresses genes that are required for plasma cell differentiation: BCL2A1, C/EBPα and MYC. As a result, LMP1 down regulation of BLIMP-1α expression diminishes the likelihood that the memory B cell will terminally differentiate into a plasma cell leading to viral lytic replication (“going viral”). This is consistent with a previous report that EBV infection of plasma cell lines leads to partial reversion to a B-cell phenotype.

In addition to providing insights into the EBV–B cell interaction, these investigations may also shed light on lymphomagenesis (see figure). The inability to exit cell cycle and terminally differentiate is a step along the path to malignancy. PRDM1 inactivating mutations have been identified in some diffuse large B-cell lymphomas and translational inhibition by cellular microRNA in others. Thus PRDM1/BLIMP1 has been identified as a tumor suppressor. Vrzalikova et al suggest...
that in diffuse large B cell lymphoma that harbor EBV, viral gene expression may contribute to lymphomagenesis by down-regulating BLIMP1.

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REFERENCES

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Megakaryocyte TLR2: immunity bullet?

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Innate immunity is the host defensive weapon against pathogens and this issue of Blood offers an interesting new bullet for the immune system gun by studying the role of Toll-like receptor 2 (TLR2) in megakaryocytic function; a field previously not explored. Beaulieu and colleagues provide evidence that inflammation, through TLR2, can increase megakaryocyte maturation and modulate its phenotype, contributing to our understanding of the interrelationship between inflammation and hemostasis.1

It is fascinating to imagine that, potentially, systemic infection could enter the bone marrow, be recognized by megakaryocytic TLR2, activate this signaling pathway to alter cellular function and, therefore, influence platelet formation.

TLRs are activated upon interaction with different pathogen-associated molecular patterns. In particular, TLR2 recognizes a large number of infectious-derived stimuli such as triacylated and diacylated lipoproteins, those from gram-negative bacteria, from mycobacteria, glycosylphosphatidylinositol anchors, phenol-soluble modulin, zymosan from fungi, glycolipids, and LPS from non-enterobacteria.2 Upon stimulation of TLR2, several different signaling proteins are stimulated, resulting in NFkB pathway activation.2 In innate immune cells, this signal cascade leads to increased expression and release of inflammatory cytokines such as interleukins and tumor necrosis factor-alpha.2 The inflammatory pattern induced by TLR2 activation is related to both immunologic and atherosclerotic disease. Atherosclerosis mouse models demonstrate an association between TLR2 and atherosclerotic lesion development.3 More interestingly, evidence supports a role for TLR2 in atherosclerosis and ischemic coronary artery disease in humans4 as well as in asthma and atopic diseases.2

There is limited information directly linking TLRs and megakaryocytes in infectious- and thrombotic-related cardiovascular diseases. TLR4, the most studied TLR until now, has been associated with hematopoietic progenitor cells and appears to contribute to increased apoptosis in myelodysplastic syndromes.5 Nevertheless, no direct association has been shown with atherothrombotic disease. However, platelet TLR2 and related innate immune transcripts have been associated with cardiovascular disease and its risk factors.6

The interplay between TLR2, inflammation, and platelets has only been partially defined. In addition to hemostasis, platelets mediate inflammation and bacterial clearance from the bloodstream. Stimulation of the immune TLR2 on the platelet surface activates phosphoinositide-3-kinase (PI3K) and causes platelet activation and platelet-dependent thrombosis.7 Inflammatory conditions are known to increase thrombopoietin (TPO) levels, thereby increasing the number of circulating platelets.7 It has been reported that TPO levels and platelet counts increase after 24 hours of exposure to low-grade endotoxin,8 a possible pathway could be related to TLR2 activation.7 Previous studies have shown megakaryocyte expression of TLRs, in particular, 1, 2, 4 and 6.1 The purpose of these receptors and the role of inflammation on megakaryocyte maturation and platelet development is still a matter of debate to which the study by Beaulieu and colleagues significantly contributes.

Beaulieu and colleagues suggest that the stimulation of megakaryocyte TLR2 by a specific, synthetic ligand, Pam3CSK4, results in an increase in phosphorylation of NFkB p65 subunit, Akt, and ERK1/2. This phosphorylation results in altered gene expression, protein levels, and megakaryocytic function. Therefore, it is hypothesized that inflammation through TLR2, can affect megakaryopoiesis, promoting increased platelet development. More specifically, Beaulieu and colleagues demonstrate that on activation of these signaling pathways factors that control megakaryocyte maturation, polyplidization, and platelet production, including transcription factors GATA-1, NF-E2, and mTOR, are up-regulated (see figure). The up-regulation of the above transcription factors is associated with megakaryocyte maturation in this model. In addition, Beaulieu and colleagues found megakaryocytic up-regulation of thrombogenic-related genes, GP1b and CD41, increased DNA content, ROS production, and adhesion to extracellular matrices. Overexpression of these inflammatory-related genes not only affects megakaryocyte/platelet development but also differentiation of surrounding cells in the bone marrow suggesting a complex interplay among inflammatory...
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