respiratory dysfunction; capillary leakiness; decreased lymphocyte counts; hypothermia or hyperthermia; and, eventually, multiple organ dysfunction.[6] This overwhelming, dysregulated systemic immune response claims millions of lives worldwide each year. Numerous surgical and nonsurgical animal models have been developed to date, including cecal ligation and puncture and lipopolysaccharide-based toxemia models. Because sepsis can occur for multiple reasons and can be aggravated by various risk factors, not a single animal model is perfect enough to recapitulate most of the clinical symptoms of sepsis. Notably, although decades and billions of dollars have been spent on these animal models, some recent studies alarm us about the uncomfortable possibility that these rodent models rather poorly mimic human inflammatory disease and thus may have seriously misled our fights against this deadly disease. This is especially true when judged by huge differences in the genomic profiles between mouse models and patients with sepsis.[7,8] This possibility haunts the troubling fact that none of nearly 150 drug candidates for sepsis tested during past decades has landed to the clinics.

Yes, mice are not humans, and yes, they are "experimental" models. Despite the substantial gap between 2 species, the current animal models have helped us tremendously to understand the disease. Moreover, considering the complex and heterogenic nature of sepsis, it would be better to have multiple animal models that could recapitulate different aspects of sepsis. In this context, the study by Jang et al provides us another useful model against this very challenging disease. Although the authors did not fully address many important features of sepsis from their mice, the findings of elevated endotoxin levels, lower lymphocyte counts, and dissolution of villous capillaries indeed warrant further studies of their mouse model. Sepsis-like phenotypes that are caused by compromised intestinal and lymph node lymphatics are quite noteworthy and possibly present some features that other mouse sepsis models have not clearly demonstrated.

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● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Nixon et al, page 2195

New insights into HIV impact on hematopoiesis

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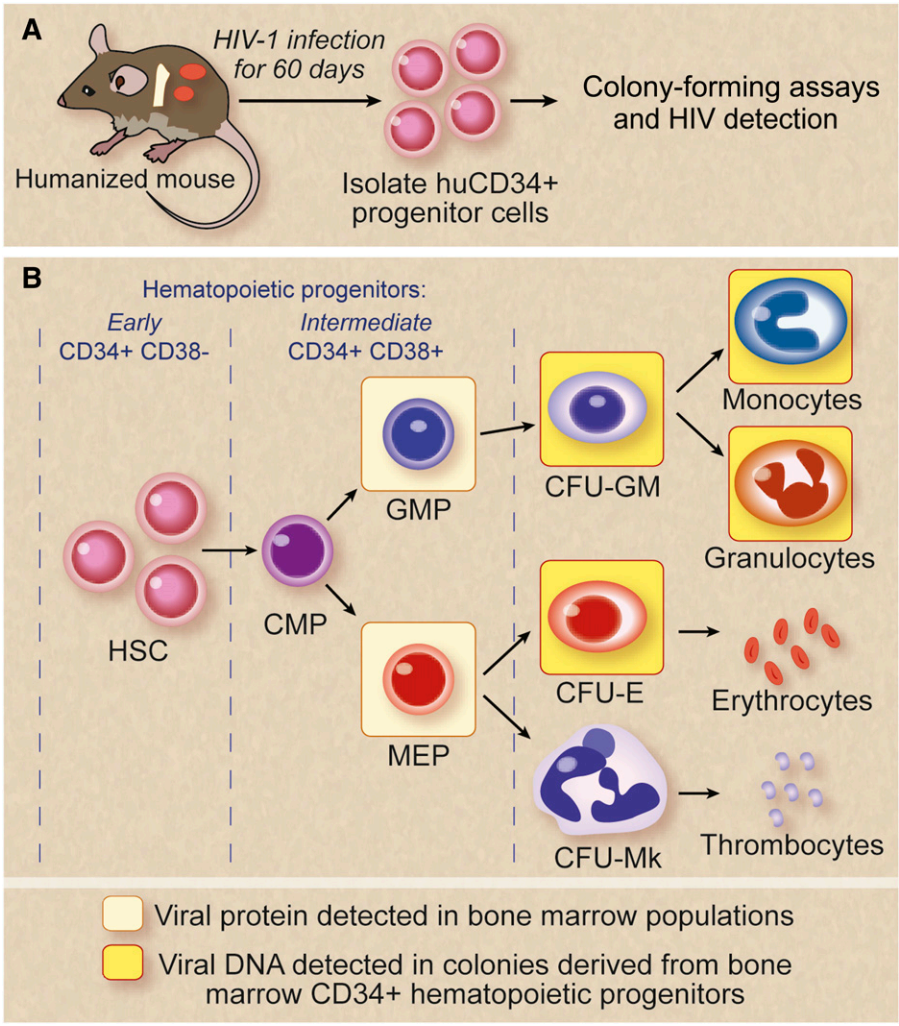
In this issue of *Blood*, Nixon and colleagues report on the impact of HIV infection on hematopoiesis. Hematopoietic stem cells (HSCs)/progenitor cells were subjected to HIV infection in vitro and in vivo using a humanized mouse model. They conclude that direct infection of intermediate progenitor cells by HIV adversely affects their hematopoietic potential, resulting in the observed cytopenias in HIV patients.[1]

Patients with long-term HIV infection often exhibit multiple hematopoietic syndromes that encompass anemia, granulocytopenia, and thrombocytopenia, suggesting a central deficiency in hematopoiesis.[2] A number of previous studies attempted to delineate the mechanism by which these conditions ensue.[3] However, clear understanding of the impairment mechanism(s) remained an intractable problem because of the paucity of studies using a suitable experimental animal model that closely recapitulates human hematopoiesis during an ongoing HIV infection in vivo.

Three main possibilities exist for the observed hematological abnormalities.[2,3] The first being the direct productive HIV infection of the early HSCs themselves with resultant deleterious effects. Although many previous studies were unable to detect HIV infection of $CD34^+$ HSCs, more recent evidence pointed to infection in at least a subset of individuals.[2-6] However, its impact other than in viral latency is unclear. Effects of HIV on intermediate progenitors were not fully evaluated until the present study. Second, even without direct productive infection, it is possible that HIV proteins such as the envelope protein and/or abnormal levels of cytokine milieu in the bone marrow (BM) of infected individuals may have indirect effects on hematopoietic progenitors, with many previous studies attesting to this. Alternatively, prolonged antiretroviral therapy in conjunction with other frequently used drugs in these patients may compromise the BM microenvironment, with resultant adverse effects on differentiating hematopoietic cells of various lineages. Accumulated evidence thus far suggests that a combination of these factors may play a role and contribute to overall hematopoietic deficiency. However, teasing out the individual contribution of each of these factors in vivo has proven to be difficult.

The studies of Nixon et al[1] exploited a humanized mouse model to extend the results seen with direct in vitro exposure/infection of $CD34^+$ HSCs and their later intermediate progenitor cells. The



HIV infection impairs HPCs. A combination of colony-forming and viral detection assays were used on in vitro– and in vivo–infected HPCs to demonstrate hematological abnormalities. Marked viral-induced suppression was seen on intermediate progenitor cells. (A) For in vivo studies, a humanized BM, liver, and thymus mouse model that supports human hematopoiesis and is susceptible to HIV infection was used. Sixty days postinfection, HPCs were examined for the presence of viral protein expression by flow cytometry and viral DNA by PCR. (B) Viral protein expression was detected in the GMP and the MEP cells. Colony assays showed HPC impairment. HIV-1 infection was detected in multiple populations of intermediate HPCs and their progeny from BM-derived cells of HIV-1–exposed mice. HIV genomes were detected in a granulocyte, macrophage, and erythroid colonies. Viral protein/DNA detection is indicated in colored boxes. CFU-E, colony-forming unit erythroid; CFU-GM, colony-forming unit granulocyte macrophage; CFU-Mk, colony-forming unit megakaryocyte. Professional illustration by Debra T. Dartez.

new-generation humanized mice derived by transplantation of human HSCs are capable of multilineage hematopoiesis and are susceptible to HIV infection showing chronic viremia and associated CD4 helper T-cell loss.[7] In these mice, the transplanted HSCs home to the BM and set up residence. Here a BM, liver, and thymus humanized mouse version was used (see figure) because it is more appropriate than the previously used severe combined immunodeficiency-hu thy/liv (SCID-hu) mice that lack the human HSC engraftment in BM.[8]

Effects of HIV infection on hematopoietic progenitor cells (HPCs) were evaluated in vivo in the absence of long-term highly active antiretroviral treatment, thus precluding the potential impact of antiretroviral drugs on hematopoiesis. Another important aspect of the current study is the systematic correlation of in vitro data with that of the results obtained from in vivo data. The first set of experiments evaluated infection of a mixed population of $CD34^+$ HSCs with an envelope glycoprotein of the vesicular stomatitis virus–pseudotyped HIV devoid of env and vpr genes, thus precluding their potential direct/indirect effects on hematopoiesis. Colony-forming unit (CFU) assays revealed both a decrease in their colony size and number. Yields of erythroid, megakaryocyte, and macrophage colonies were found to be negatively impacted. Subpopulations of intermediate HSCs—common myeloid progenitor (CMP) and granulocyte-monocyte progenitor (GMP) but not megakaryocyte-erythroid progenitor (MEP)—were shown to coexpress HIV co-receptors, either CCR5 or CXCR4 together with the primary receptor CD4. Infection of purified populations of CMP, GMP, and MEP with a dual-tropic HIV resulted in infection of these cells (albeit a small proportion), indicating their virus susceptibility. MEPs not coexpressing CD4 with either CCR5 or CXCR4 were found to be even more infection-prone however, suggesting CD4-independent entry that needs to be further investigated. In the in vivo experiments, $CD34^+$ cells isolated from HIV-infected humanized mice with 3 different viral strains were positive for viral sequences by polymerase chain reaction (PCR) irrespective of viral tropism. When $CD34^+$ cells isolated from these infected mice BM were evaluated by CFU assays, generation of all lineages were found to be adversely affected, erythroid cells in particular, with the exception of granulocytes. In vivo–infected $CD34^+$ cells showed full-length viral DNA, demonstrating that these cells (although impaired) do survive and give rise to colonies in vitro, albeit smaller (see figure).

Although these studies made a good start in developing an experimentally amenable in vivo system, several critical questions remain and further studies are needed to validate this system further. (1) Are the respective cell lineages harboring the proviral DNA productively infected and contribute to the spread the virus? (2) Why are certain lineages more profoundly affected than others and what is the mechanism? (3) The current studies are performed with a limited number of viral clones representing only a few viral variants; therefore, how does the infection affect HPCs with patient-derived primary isolates (consisting of viral swarms with mutated sequences) from early stages vs late stages of infection when the hematological abnormalities are more severe? It is essential that results obtained from these mouse studies be verified by comparing them with those from the HIV-infected individuals at different stages of the disease. For example, it needs to be determined if intermediate hematopoietic progenitor cell populations obtained from

highly active antiretroviral treatment-naive patients harbor HIV provirus and are consequently impaired in their development.

In summary, these studies broke new ground and established that certain intermediate hematopoietic cells are virus-susceptible during an ongoing HIV infection in vivo, resulting in their impairment. In addition, this work also demonstrated the utility of a humanized mouse model to further evaluate important questions on HIV-mediated hematological abnormalities.

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● ● ● LYMPHOID NEOPLASIA

Comment on Nogai et al, page 2242

Shaping oncogenic NF-κB activity in the nucleus

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In this issue of *Blood*, Nogai et al have identified the atypical nuclear factor–κB (NF-κB) modulator IκBζ as a key factor that drives oncogenic NF-κB activity in an activated B-cell subtype of diffuse large B-cell lymphoma (ABC DLBCL).[1]

Sustained activation of NF-κB survival signaling is a hallmark of ABC DLBCL. Previous work has largely focused on the identification of oncogenic mutations and molecular mechanisms in the cytoplasm that contribute to deregulated activation of the NF-κB signaling pathway.[2] By identifying nuclear IκBζ (also termed MAIL) as an essential survival factor in the majority of ABC DLBCL cells, the current study of Nogai, Lenz, and colleagues highlights the importance of also shaping an oncogenic transcriptional NF-κB response inside of the nucleus.[1]

The NF-κB family comprises the subunits NF-κB1/p50, NF-κB2/p52, p65/RelA, c-Rel, and RelB that can form various homodimers and heterodimers with distinct biological properties.[3] In contrast to the cytosolic NF-κB inhibitors IκBα, IκBβ, or IκBε, the atypical IκB protein IκBζ is regulating NF-κB exclusively in the nucleus. After stimulation of innate immune or cytokine receptors in macrophages, IκBζ expression is induced and it activates a subset of NF-κB target genes including *IL-6* by selectively enhancing transcriptional activity of p50 homodimers.[4,5] In this issue, the authors demonstrate that IκBζ is highly expressed in the majority of ABC DLBCL cell lines and patient samples and contributes to the induction of the NF-κB target gene signature (see figure).[1] IκBζ depletion induces toxicity in ABC DLBCL cells, but not in other NF-κB–dependent or –independent lymphomas. Mechanistically, IκBζ promotes transcriptional activity of p50 and p52 homodimers; in ABC DLBCL cells, IκBζ binds exclusively to the p50 and p52 subunits of NF-κB. Because p50 or p52 do not contain transcriptional activation domains, the data suggest that IκBζ confers transactivating potential to allow induction of a large number of NF-κB target genes in ABC DLBCL cells (see figure). Also, it is conceivable that IκBζ may facilitate recruitment of transcriptionally active NF-κB heterodimers by displacing inactive p50 and p52 homodimers. In any case, the strong effects on ABC DLBCL viability after knockdown underscore that IκBζ is a key driver of pathological NF-κB transcription inside of the nucleus.

As IκBζ does not confer catalytic activity, it may not be a direct target for pharmacologic inhibition. Nevertheless, the data suggest that strategies to reduce IκBζ protein levels could be beneficial for ABC DLBCL therapy. Emphasizing this notion, the study reveals interesting insights into the regulation of IκBζ expression in the tumor cells (see figure). Previous data demonstrated that IκBζ is not present in resting cells, but expression is highly induced upon stimulation of Toll-like receptors or interleukin 1 (IL-1) receptor by a myeloid differentiation protein 88 (MYD88)– and IL-1 receptor-associated kinase 4 (IRAK4)–dependent pathway.[5,6] Congruent with a critical function of canonical NF-κB signaling, oncogenic MYD88 or caspase recruitment domain-containing protein 11 (CARD11) variants trigger IκBζ expression in ABC DLBCL.[1] However, constitutive NF-κB activation may not be sufficient for full IκBζ induction because IκBζ expression is low in Hodgkin lymphoma or multiple myeloma, despite the fact that survival of both lymphomas relies on NF-κB. Interestingly, signal transducer and activator of transcription 3 (STAT3) activation can also induce the expression of IκBζ.[7,8] In ABC DLBCL, STAT3 is phosphorylated and activated by an autocrine loop that involves the secretion of the cytokines IL-6 and IL-10 (see figure).[2] Importantly, expression of both cytokines is under control of IκBζ and, consequently, IκBζ knockdown severely diminishes STAT3 phosphorylation.[1] Even though it remains to be shown whether STAT3 also directly influences IκBζ in ABC DLBCL, the data imply that IκBζ, IL-6/IL-10, and STAT3 could constitute a vicious autoregulatory feed-forward cycle that contributes to the