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PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

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STIM1 for stimulation of phagocyte NADPH oxidase

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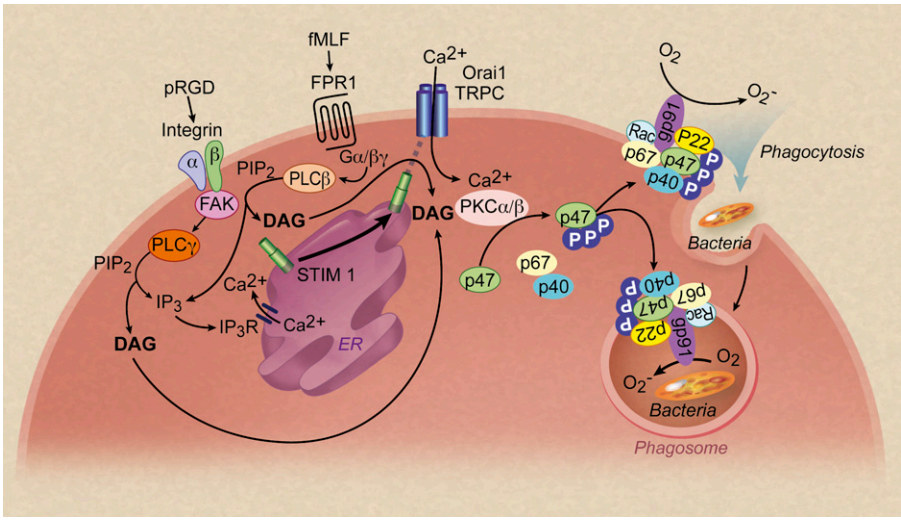
In this issue of *Blood*, Zhang et al show that mice lacking the stromal-interacting molecule 1 (STIM1) gene in bone marrow cells are more susceptible to bacterial infection but are resistant to ischemia/reperfusion injury because of defective activation of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[1]

The concentration of cytosolic Ca^{2+} is crucial for important cellular functions such as neurotransmitter release, muscle contraction, and transcriptional regulation.[2] A variety of cell surface receptors signal through phospholipase C activation for the generation of inositol 1,4,5-trisphosphate (IP_3) that triggers Ca^{2+} release from endoplasmic reticulum (ER), a major intracellular Ca^{2+} store. The resulting store-operated Ca^{2+} entry (SOCE) is responsible for sustained increase of cytosolic Ca^{2+} concentration resulting from the opening of membrane channels and influx of Ca^{2+}, which is present extracellularly at higher concentrations. At the center of this regulatory process is STIM1, a protein that senses Ca^{2+} concentration changes in the ER and communicates with calcium channels on plasma membrane (see figure; reviewed in Baba and Kurosaki[3]).



Activation of the formyl peptide receptor 1 (FPR1) by fMet-Leu-Phe (fMLF), the integrin by pRGD (an Arg-Gly-Asp-containing fibronectin-like binding fragment), and the Fcg receptors (not shown) leads to phospholipase C (PLC) activation, hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), and IP_3 production. The resulting release of Ca^{2+} from ER triggers STIM1-dependent Ca^{2+} influx, causing sustained increase of intracellular Ca^{2+} concentration through the opening of the ORAI calcium release-activated calcium modulator, and the transient receptor potential (TRP) channel, TRPC. Ca^{2+}-dependent and diacylglycerol (DAG)-dependent activation of PKCa and PKCb leads to phosphorylation of $p47^{phox}$ (circled P's in the figure) and assembly of a functional NADPH oxidase in neutrophils. FAK, focal adhesion kinase that mediates integrin signaling; $p47^{phox}$, a cytosolic component of the phagocyte NADPH oxidase (phox) with an apparent molecular weigh of 47 kDa. Professional illustration by Debra T. Dartez.

Zhang et al provide experimental evidence for an in vivo function of STIM1 in neutrophil-mediated killing of bacteria and ischemia/reperfusion injury.[1] Through bone marrow transplantation with *Stim1*$^{-/-}$ fetal liver cells, the authors successfully obtained mouse neutrophils lacking STIM1, thus overcoming lethality resulting from *Stim1* deletion. The availability of these chimeras has allowed the authors to examine the functional changes resulting from STIM1 deficiency. One of their experiments shows that these mice are prone to infection by *Listeria monocytogenes* and *Staphylococcus aureus*, resulting in more pronounced pathologic changes in the lungs. The increased susceptibility can be attributed to diminished neutrophil superoxide production, because phagocytosis and neutrophil infiltration are minimally altered while neutrophil NADPH oxidase activation is abrogated. These findings are consistent with a previous report on the involvement of STIM1 in NADPH oxidase activation using neutrophil-like HL-60 cells.[4] The study by Zhang et al further demonstrates that SOCE is defective in *Stim1*$^{-/-}$ neutrophils activated through chemoattractant receptors as well as Fcγ receptors and integrins. These receptors are activated in different manners, but they all stimulate IP_3 production that leads to SOCE (see figure). Zhang et al also show that sustained increase in cytosolic Ca^{2+} concentration is required for neutrophil superoxide production primarily because of the role of calcium in the activation of the classical protein kinase C (PKC) isoforms (PKCα and PKCβ in neutrophils).

These PKCs are highly important to the phosphorylation of the phagocyte NADPH oxidase components, $p47^{phox}$ and $p40^{phox}$. Phosphorylation of $p47^{phox}$ is of particular interest because this posttranslational modification changes the conformation of $p47^{phox}$, leading to its membrane translocation and binding to $p22^{phox}$, a subunit of the core NADPH oxidase termed flavocytochrome b_{558}.[5] In STIM1-deficient neutrophils, PKC-dependent phosphorylation of these cytosolic factors of the NADPH oxidase is markedly reduced.

The work by Zhang et al provides molecular details for STIM1-dependent Ca^{2+} influx leading to phagocyte NADPH oxidase activation and also shows several interesting aspects of Ca^{2+} signaling in relation to other neutrophil functions. It is particularly interesting that neutrophil chemotaxis, known to rely on Ca^{2+} influx,[6] is not affected by STIM1 deficiency, suggesting that a sustained rise in cytosolic Ca^{2+} may not be required. It is possible that other forms of Ca^{2+} signaling, including transient Ca^{2+} mobilization from intracellular stores,[7] or localized calcium flickers as reported in other types of cells,[8] may be sufficient for neutrophil migration. Chemotaxis mediated by different receptors may also have different requirement for Ca^{2+} influx.[9]

Production of oxygen radicals is a major bactericidal function of phagocytes. However, it is a double-edged sword because of its tissue-damaging property. Zhang et al show that the *Stim1*$^{-/-}$ chimeras are resistant to ischemia/reperfusion injury to the liver, suggesting that neutrophil production of oxygen radicals might contribute to the pathological changes in wild-type subjects. Future work using granulocyte-specific deletion of *Stim1* will be helpful to ascertain the respective contribution of different blood cells to the observed changes. These studies create new opportunities for therapeutic intervention that targets STIM1.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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LYMPHOID NEOPLASIA

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CLLonal selection: survival of the fittest?

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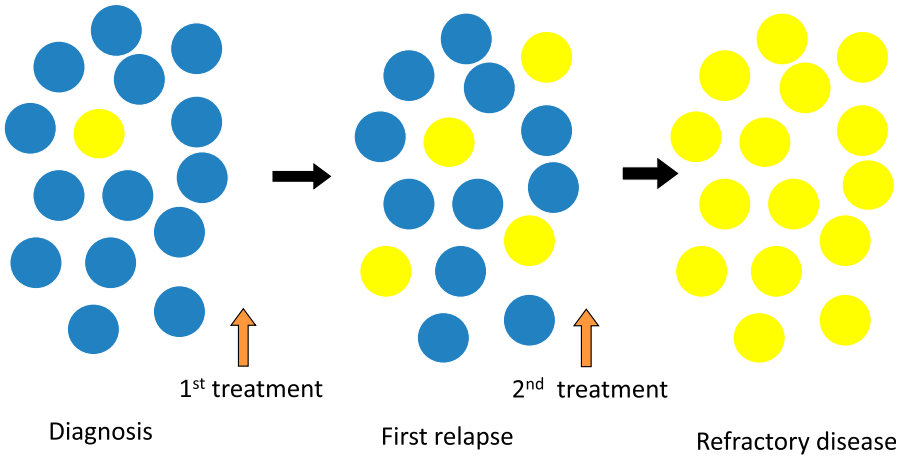
In this issue of *Blood*, Rossi et al provide further evidence for clonal evolution in chronic lymphocytic leukemia (CLL) and demonstrate the clinical importance of small *TP53*-deleted subclones detected at diagnosis in determining the natural history of the disease.[1]

The basic principle of Darwinian evolutionary theory is the natural selection of the fittest variants. Fitness is defined by the ability to survive and reproduce and the "fittest" as those best adapted to achieve this. This concept of subclonal selection of the "fittest" variants was first applied to cancer by Nowell in 1976[2] and has subsequently been supported by modern genomics.[3] Knowledge of the clonal diversity and clonal selection operating in any specific cancer is critical to the understanding of disease progression, response to treatment, and development of resistance. The clonal architecture of any cancer is in a constant state of evolutionary change, which can take place over prolonged periods. Clinically important mutations may be present at an early stage of the disease but only become evident over time through selective pressure.

The clinical importance of chromosomal abnormalities in CLL has been recognized since the late 1990s.[4] Since then, more refined techniques such as fluorescence in situ hybridization (FISH) and Sanger sequencing



Clonal selection and expansion of *TP53*-mutated subclones during the clinical course of CLL. Yellow circles indicate the *TP53*-mutated CLL cell which expands through the disease course from below the level of conventional detection methods at diagnosis to become the dominant population in refractory disease. Blue circles indicate the non-*TP53*–mutated CLL cell which is the dominant clone at diagnosis but is subsequently replaced by the *TP53*-mutated subclone.