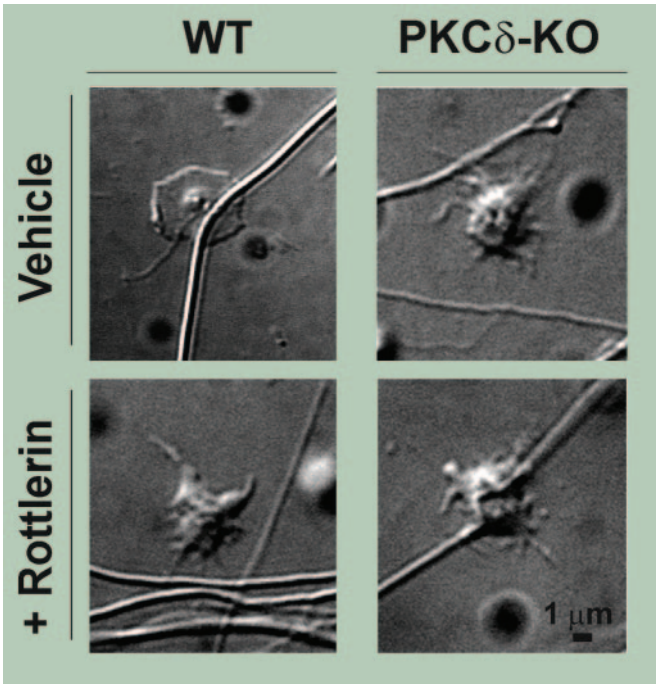

PKCδ negatively regulates filopodia formation in human platelets. See the complete figure in the article beginning on page 4035.

new ideas and will inspire new studies investigating how association of VASP and PKCδ inhibit phosphorylation of VASP on Ser157. The evidence presented strongly suggests that the PKCδ activity is necessary for the observed negative regulation.

Future studies aimed at (1) the stoichiometry of PKCδ interaction with VASP, (2) how PKCδ activity is involved in inhibiting filopodia formation, (3) whether PKCδ activation is required for its interaction with VASP, (4) whether steric hindrance or conformational change resulting from PKCδ binding is responsible for decreased VASP phosphorylation on Ser157, and (5) whether PKCδ is constitutively associated with VASP in resting platelets or whether it becomes associated upon stimulation of platelets will be very interesting. Thus, this work, using a genetic approach, provides an intriguing avenue for further studies that might aid in design and development of novel therapeutic agents for the treatment of thrombotic disorders.

The author declares no competing financial interest. ■

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RED CELLS

Comment on Inoue et al, page 4232

How many mutations does it take to get PNH?

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One of the puzzles in understanding paroxysmal nocturnal hemoglobinuria is the reason for the expansion of the defective clone. In this issue, Inoue and colleagues suggest that a second mutation might be needed.

The characteristic biochemical lesion in paroxysmal nocturnal hemoglobinuria (PNH) is the clonal lack of glycosyl phosphatidylinositol (GPI)–linked proteins on the membrane of the affected blood cells. This lesion accounts for many of the clinical manifestations of the disease, particularly intravascular hemolysis and its consequences and, probably, the marked propensity for venous thromboses. The lesion results from mutations of the gene, called *PIGA*, that codes for one of the components necessary for the biosynthesis of the GPI anchor.

Hematopoietic precursors with defects in this gene have been identified in many if not most healthy donors tested, so it is clear that this mutation is not enough to account for the occurrence of the disease. The PNH clone must be selected and expanded in order for a sufficient number of blood cells to be present to cause clinical manifestations. It has been suggested that the clone is selected for by the immunologic processes underlying aplastic anemia (the Luzzatto-Young hypothesis) and, indeed, a large proportion of patients with aplastic anemia exhibit the cells characteristic of PNH, albeit usually in relatively small proportions. In only a minority of such patients does the clone expand to clinical relevance. Thus, neither the characteristic biochemical abnormality nor the selection process explains requisite expansion of the clone in those patients with PNH.

In their paper, Inoue and colleagues offer a possible insight into this problem. In 2 patients, the authors have found a rearrangement of chromosome 12 that has apparently induced a mutation causing the "deregulation" of the *HMGA2* gene. This gene produces a protein, a member of the high-mobility group of proteins, that functions as an architectural transcription factor, promoting transcription by facilitation of the assembly of transcription factors into an "enhanceosome." Mutations causing deregulation of the gene have been found in benign mesenchymal tumors. Inoue et al argue that in these 2 patients, overactivity of the *HMGA2* gene as a consequence of the chromosomal break gives the selected *PIGA*–mutated clone the expansion that it needs to become clinically manifest but not the uncontrolled expansion of a malignancy. Thus, PNH is viewed as a benign tumor of the bone marrow involving the selected GPI-deficient clone.

This paper's importance is that it points to an area of study that may solve one of the 2 basic problems in understanding PNH: the basis of clonal selection and the basis of clonal expansion. Most patients do not have chromosomal abnormalities, and it will be difficult to know where to look for second mutations that

are not manifest in such gross ways, but at least the results in these 2 patients suggest a starting point. When more is known, my guess is that the relationship of PNH to myelodysplastic syndromes and to acute leukemia will also be adumbrated.

The author declares no competing financial interests. ■

PHAGOCYTES

Comment on Renshaw et al, page 3976

Fluorescent neutrophils throw the spotlight on inflammation

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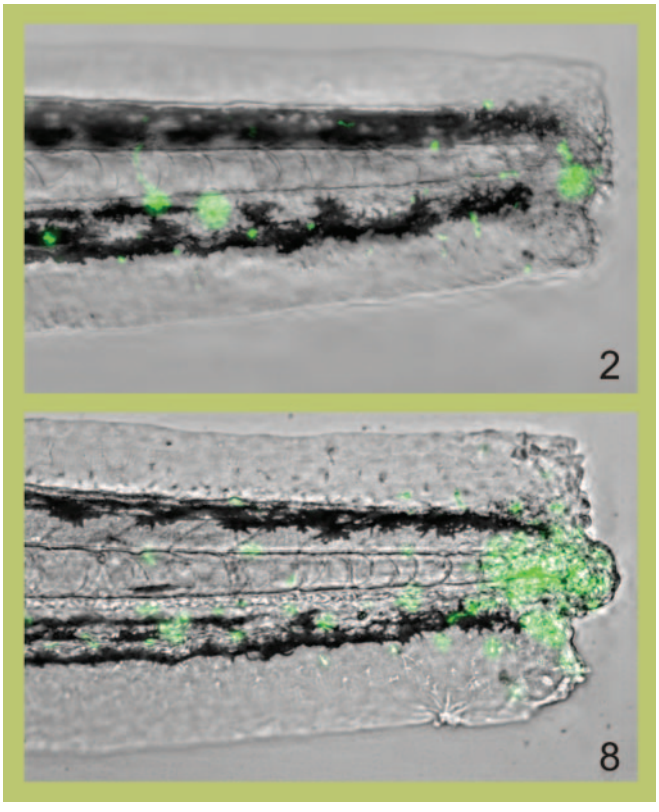
The generation of transgenic zebrafish with fluorescent neutrophils provides a new tool to study the dynamic contribution of granulocytes to acute inflammation and its pathological consequences.

Inflammation can only be studied in toto using in vivo models, primarily because of the diverse and dynamic cellular processes involved. Vascular integrity is secured, endothelial cells are activated, neutrophils (initially) and macrophages (later) egress from the circulation and localize to the inflammatory focus, and the adaptive immune system responds to foreign agents. Once the whole act is initiated, it must then resolve appropriately, or else pathological tissue damage will occur. While the individual molecular and cellular components of acute inflammation have been studied (eg, cytokine and chemokine biology, endothelial activation, maintaining the supply of phagocytes and their activation, chemotaxis), there is still much to be learned about the integrated process.

Animal models are required for this. In this issue of *Blood*, Renshaw and colleagues report the generation of a transgenic zebrafish expressing enhanced green fluorescence protein (EGFP) from the zebrafish myeloperoxidase promoter, resulting in fluorescent neutrophils. The optical transparency of whole zebrafish embryos means that fluorescent neutrophils can be followed in real time in vivo, and Renshaw and colleagues elegantly demonstrate how acute inflammation following a standardized experimental trauma (tail transection) can be followed in real time and quantitated, and the effects of pharmacological manipulation assessed (see figure). Renshaw et al used BAC recombination to construct a transgene placing EGFP under control of 130 kb of upstream regulatory sequence; very recently, another group has also reported the construction of a myeloperoxidase-promoter:EGFP-transgenic zebrafish, but using 8 kb of regulatory sequence.[1] While both lines are very similar, that of Mathias et al[1] shows early expression elsewhere in the tail, which may indicate that all the myeloperoxidase regulatory elements that restrict expression to leukocytes are not present in the smaller promoter or, alternately, may reflect a transgene integration site effect. Collectively, these 2 reports have already enriched our knowledge of the dynamics of acute inflammation, presenting new evidence that 2 mechanisms contribute to its resolution: neutrophil apoptosis (Renshaw



Progressive accumulation of fluorescent neutrophils (green) at 2 and 8 hours after transection of the tail of a zebrafish larva. See the complete figure in the article beginning on page 3976.

Transgene (*promoter:*fluorescent marker)	Cell type marked	Reference
myeloperoxidase [130kb]:EGFP	neutrophils primarily	Renshaw et al
myeloperoxidase [8kb]:EGFP	neutrophils primarily, some monocyte lineage cells	1
fli1:EGFP	endothelial cells, macrophages	2, 6
cd41:EGFP	thrombocytes	7
rag2:EGFP	lymphocytes	8
pu.1[5.3kb]:EGFP	early leukocytes	9
pu.1[9kb]:EGFP	early leukocytes	10

Examples of stable transgenic zebrafish lines with fluorescent cell types involved in actue inflammation. Illustration by A. Y. Chen.