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

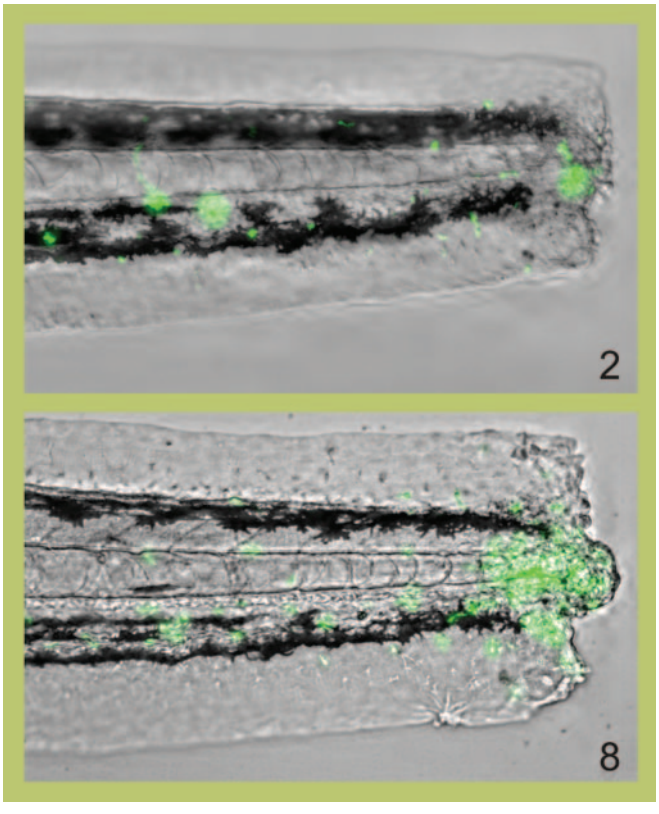
Comment on Renshaw et al, page 3976

Fluorescent neutrophils throw the spotlight on inflammation

Graham J. Lieschke WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

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.



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.

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

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.

et al) and their active departure from the inflammatory site.[1]

These new transgenic zebrafish are a welcome addition to the now impressive range of zebrafish with fluorescently marked cell types relevant to inflammation (see table). The involvement of fluorescent embryonic macrophages in acute inflammation has been studied in zebrafish with a *fli1*-driven EGFP,[2] and the same line has been used to study endothelial regeneration after wounding.[3] Although the collective data indicate that modeling inflammation in zebrafish is relevant to human inflammatory disease, several details require further exploration. There is some evidence that myeloperoxidase may not be a totally specific marker for zebrafish neutrophils, but may mark some early macrophage-lineage cells as well.[1] At an ultrastructural level, zebrafish myeloperoxidase-containing neutrophil primary granules have a markedly different morphology from those of mammalian neutrophils[4]; whether this is reflected in a functional difference remains to be determined. It has also not been formally demonstrated whether zebrafish neutrophils are phagocytic.

Transgenic mice with EGFP-marked leukocytes driven from the lysozyme promoter are available[5] and have been used to study leukocyte distribution in vivo. However, zebrafish offer unique opportunities to investigate the regulation of these complex cellular processes genetically by exploiting these fluorescently-marked lines in efficient, affordable forward genetic screens, and for identifying new therapeutic lead compounds modifying cellular contributions to acute inflammation by high-throughput chemical screening. With the spotlight now on inflammation in zebrafish, we can hope for new insights into its genetic regulation and, hopefully, the identification of novel anti-inflammatory therapeutic agents.

The author declares no competing financial interests.

REFERENCES

1. Mathias JR, Perrin BJ, Liu TX, Kanki J, Look AT, Huttenlocher A. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. J Leukoc Biol. Prepublished on September 8, 2006, as DOI:10.1189/jlb.0506346.

2. Redd MJ, Kelly G, Dunn G, Way M, Martin P. Imaging macrophage chemotaxis in vivo: studies of microtubule function in zebrafish wound inflammation. Cell Motil Cytoskeleton. 2006;63:415-422.

3. Huang CC, Lawson ND, Weinstein BM, Johnson SL. reg6 is required for branching morphogenesis during blood vessel regeneration in zebrafish caudal fins. Dev Biol. 2003;264:263-274.

4. Lieschke GJ, Oates AC, Crowhurst MO, Ward AC, Layton JE. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. Blood. 2001;98:3087-3096.

5. Faust N, Varas F, Kelly LM, Heck S, Graf T. Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. Blood. 2000;96:719-726.

6. Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. Dev Biol. 2002;248:307-318.

7. Lin HF, Traver D, Zhu H, et al. Analysis of thrombocyte development in CD41-GFP transgenic zebrafish. Blood. 2005;106:3803-3810.

8. Langenau DM, Traver D, Ferrando AA, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299:887-890.

9. Ward AC, McPhee DO, Condron MM, et al. The zebrafish spi1 promoter drives myeloid-specific expression in stable transgenic fish. Blood. 2003;102:3238-3240.

10. Hsu K, Traver D, Kutok JL, et al. The pu.1 promoter drives myeloid gene expression in zebrafish. Blood. 2004;104:1291-1297.

HEMATOPOIESIS

Comment on Allman et al, page 4025

Plasmacytoid DCs fail to soar without Ikaros

Marco Colonna WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

The developmental pathway of plasmacytoid DCs (pDCs) is poorly understood. In this issue of *Blood*, Allman and colleagues break new ground by demonstrating that the transcription factor Ikaros is crucial for terminal differentiation of pDCs.

Plasmacytoid dendritic cells (pDCs) specialize in secreting high amounts of type I interferons (IFNα and IFNβ) in response to DNA and RNA viruses. Because they share some attributes of conventional DCs (cDCs), particularly MHC class II expression, pDCs are also considered a DC subset capable of antigen presentation. Thus, pDCs are thought to be crucial for initiating antiviral innate responses as well as jump-starting adaptive responses.

Although pDCs were identified some time ago, their origin, development, and lineage relationship with other cells of the immune system remain only partially defined. It has been clearly established that Flt-3 ligand (Flt-3L) directs the development of pDCs from hematopoietic stem cells (HSCs) in the bone marrow.[1] However, whether pDCs develop as a branch of the lymphoid or myeloid lineage is unclear, as pDCs have been reported to differentiate from the common lymphoid progenitor, the common myeloid progenitor, or both. These observations led to the suggestion that pDC development is more flexible than that of classical myeloid and lymphoid cells.

Strong evidence for developmental plasticity has been provided by studies aimed at defining the transcriptional program driving the development of pDCs from HSCs; collectively, this work has identified multiple transcription factors previously implicated in either lymphoid or myeloid development. The transcription factor Spi-B promotes pDC and B-cell development over that of T cells,[1] whereas IFN regulatory factor 8 (IRF-8; also called ICSBP), a critical transcriptional factor for the myeloid cell lineage, is required for the development of both pDCs and $CD8\alpha^+$cDCs.[2,3] pDC development is negatively regulated by members of the basic helix-loop helix (bHLH) family, which also block development of B and T cells, sparing the development of cDCs and natural killer (NK) cells.[1]

The transcription factor Ikaros is required for the development of multiple hematopoietic lineages[4] and has also been implicated in pDC development. Among various Ikaros mutant mice that have been generated, a null mutation prevented development of the $CD8\alpha^-$ cDC subset, while a dominant-negative mutation resulted in lack of both $CD8\alpha^+$ and $CD8\alpha^-$ cDCs.[5] In the present study, Allman and colleagues analyzed a mutant mouse line ($Ik^{L/L}$) expressing a truncated Ikaros protein that functions normally but is expressed at very low levels. Allman et al showed that $Ik^{L/L}$ mice lack peripheral pDCs, but not cDCs. Remarkably, $Ik^{L/L}$ bone marrow does contain a pDC population that appears to be blocked at an early stage of differentiation characterized by low levels of B220 and lack of the cell surface receptor Ly-49Q. Moreover, $Ik^{L/L}$ bone marrow cells respond to