third isomorph of the protein, with a transcript likely beginning in the terminal repeats themselves. As such, the encoded protein lacks epitopes recognized by extant monoclonal antibodies and is missed by many of the PCR primer pairs used to characterize EBV gene expression. The authors term the new protein LMP2-TR.

Success with any or all of the strategies targeting LMP2-TR in extranodal NK/T lymphoma may mean that the discovery of the new protein isomorph, rather than merely a footnote to the gene expression map of EBV, will begin an interesting new chapter in the book defining immunotherapeutic targets in EBV-associated malignancies.

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

REFERENCES


Comment on Balla et al, page 3944

Eosinophils are in the swim!

Helene F. Rosenberg NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

The freshwater tropical fish, Danio rerio, has most certainly arrived. A species previously familiar only to aquarium enthusiasts, the zebrafish has moved into a central role as a unique and flexible model for the study of vertebrate biology, in fields including embryology and neurobiology, and, more recently extending to hematopoiesis, immunology, and infectious disease.1 2

In this issue of Blood, Balla et al3 present a detailed examination of zebrafish eosinophils, an enigmatic leukocyte lineage whose role in promoting homeostasis and host defense remains uncertain despite years of research with more traditional human, mouse, and guinea pig model systems.4 5 Among the highlights of this article is an exquisite atlas of zebrafish eosinophil morphology; the authors document the isolation of gata2-expressing cells from whole-kidney marrow, coloration with standard cytology stains, and prevalence of eosinophilic myelocytes, metamyelocytes, bands, and polymorphonuclear forms with both light and electron microscopic views. In an interesting contrast to mammalian biology, the zebrafish polymorphonuclear eosinophils are found only rarely, making their discovery worthy of further consideration.

Will zebrafish stand ahead of mice as the new organism of choice for studies of eosinophil-mediated function in health and disease? Balla and colleagues6 clearly demonstrate that zebrafish eosinophils are not only readily recognizable, but also maintain important functional features. For instance, zebrafish eosinophils, similar to their human counterparts, degranulate in the response to appropriate challenge. However, several findings in the article introduce some question as to exactly what might be found in the zebrafish eosinophil granules. The authors report eosinophil-specific transcription of dr-RNase-26—an ortholog of the divergent human and mouse eosinophil ribonucleases, and 1 of the 3 RNase A ribonucleases encoded in the zebrafish genome. Yet ortholog(s) of eosinophil major basic protein (MBP), a highly conserved secretory granule protein to which many of the current functions of eosinophils are attributed, have not been detected. As such, what exactly is inside the zebrafish eosinophil granule? Are proinflammatory cytokines more prominent in zebrafish than they are in the mammalian eosinophil granules? Lee and Lee7 have argued that release of cationic granule proteins is not as crucial physiologically as it has been perceived to be historically; perhaps the zebrafish model will permit us to evaluate this hypothesis directly (see figure).

The authors also demonstrate peripheral eosinophilia in response to allergen challenge.

Transmission electron micrographs of eosinophils isolated from the spleen of an IL-5–transgenic mouse.8 Note the structural similarities shared with the zebrafish eosinophils isolated from the whole-kidney marrow displayed in micrographs shown in Figure 2 of the Balla et al article.9 Notable features include an eccentric, indented nucleus and irregularly shaped electron-dense cytoplasmic granules (left panel: ×7000 magnification; right panel: ×10 000 magnification). Micrograph prepared by Dr Elizabeth R. Fischer (Research Technologies Branch, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health [NIAID, NIH]) and provided by Dr Kimberly D. Dyer (Laboratory of Allergic Diseases, NIAID, NIH).
and similarly, accumulation of eosinophils in tissues in response to parasitic infection. Nonetheless, it is not at all clear what promotes this response at the biochemical level. In mammalian biology, eosinophilia in response to these stimuli is directly dependent on the actions of the Th2 cytokine, interleukin-5 (IL-5), which promotes eosinophil colony expansion, eosinophil priming, and prolonged eosinophil survival in the periphery. Similar to what was described above for MBP, analysis of the zebrafish genome has not revealed any sequences orthologous to mammalian IL-5, nor any that are related to its unique receptor, although other Th2 cytokine and cytokine receptor sequences have been identified, as have zebrafish orthologs of eotaxin (CCL11), a chemokine with unique eosinophil chemotaxant properties.

In summary, Balla and colleagues have provided us with an important new perspective on eosinophil hematopoiesis and a fresh start from which to examine the role of eosinophils in eliciting disease and promoting homeostasis. If the sea hath fish for every man, those of us working in eosinophil biology are certainly happy to know about Damio rerto.

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

REFERENCES

THROMBOSIS & HEMOSTASIS

Comment on Dayananda et al, page 3990

VWF self-association: more bands for the buck

José A. López and Dominic W. Chung Puget Sound Blood Center; University of Washington

In this issue of Blood, Dayananda and coworkers demonstrate that fluid-phase VWF can bind homotypically to platelet VWF, strengthening platelet-platelet association and enhancing platelet activation.

The hemostatic function of von Willebrand factor (VWF) depends on its degree of multimerization, which results from posttranslational assembly of the nascent VWF chains in the endoplasmic reticulum and Golgi apparatus. Intracellular assembly is promoted by the VWF propeptide, a 741-amino acid sequence at the N-terminus of the nascent VWF polypeptide that acts as a disulfide isomerase to promote the formation of unique disulfide bonds between VWF dimers to form an array of VWF multimers that are stored in endothelial Weibel-Palade bodies or platelet α-granules. These newly synthesized multimers can be extremely large (ultra-large VWF [ULVWF]) and hyperadhesive. Upon their secretion into the blood and under shear stress, ULVWF multimers are proteolytically cleaved by the plasma metalloprotease ADAMTS13 to produce a population of VWF multimers with lower reactivity for platelets that efficiently support primary hemostasis. Over the past several years, it has become increasingly appreciated that another important mechanism exists for regulating the hemostatic activity of VWF: its ability to self-associate. This phenomenon was first reported by Savage et al who showed that fluid-phase VWF multimers could homotypically associate with VWF multimers that were immobilized onto a collagen surface; the self-associated VWF multimers supported platelet adhesion under shear stress. Fluid-phase VWF lacking either the A1 or the A3 domain still bound immobilized wild-type VWF; later studies showed that VWF self-association involved multiple domains and—in the case of plasma VWF binding to ULVWF attached to an endothelial surface—required free thiols in plasma VWF. The self-association has been reported to be reversible, although both studies examined this issue indirectly. VWF self-association appears to be facilitated by unfolding of the molecule, as it is increased by both shear stress and ristocetin. One apparent consequence of VWF self-association is an increased local density of platelet-binding sites, resulting in enhanced platelet adhesion and surface coverage.

The structure of self-assembled VWF multimers was characterized by immunofluorescence microscopy by Barg et al who showed that purified VWF multimers assembled into a macroscopic network of cross-linked fibers on a collagen surface under flow. This network of VWF fibers bound platelets under shear stress and was degraded by ADAMTS13 and other plasma proteases. Similarly, ristocetin, which mimics the effect of shear stress on VWF molecules, also promoted self-association of VWF multimers into a network of fibers in solution.

In this issue, Dayananda et al demonstrate that soluble VWF can associate with VWF bound to GPIbα on platelets. VWF multimers lacking the A1 domain (ΔA1-VWF) homotypically associated with normal plasma VWF molecules on GPIbα on the platelet surface in a shear-dependent manner. Assembly of multiple VWF molecules on the platelet surface increases the magnitude of tensile force exerted on the platelet through GPIbα, which triggers platelet activation. These novel findings suggest that dynamic self-association of VWF under shear stress not only promotes initial platelet adhesion at the site of vascular injury, but may also contribute to platelet aggregation and pathological thrombus growth, particularly in response to inflammation or under abnormal flow conditions. They also raise the possibility that augmented or acceler-
Eosinophils are in the swim!

Helene F. Rosenberg