methylation-iPLEX forecast the natural history of chronic lymphocytic leukemia. *Blood*. 2019;134(8):688-698.

7. Nadeu F, Royo R, Clot G, et al. IGLV3-21R110 identifies an aggressive biological subtype of chronic lymphocytic leukemia with intermediate epigenetics. *Blood*. 2021;137(21):2935-2946.

8. Maity PC, Bilal M, Koning MT, et al. IGLV3-21*01 is an inherited risk factor for CLL through the acquisition of a single-point mutation enabling autonomous BCR signaling. *Proc Natl Acad Sci U S A*. 2020;117(8):4320-4327.

9. Scarfo L, Ghia P. What does it mean I have a monoclonal B-cell lymphocytosis? Recent insights and new challenges. *Semin Oncol*. 2016;43(2):201-208.

10. Rosenquist R. Time to include IGLV3-21R110 status in CLL prognostication? *Blood Adv*. 2023;7(23):7382-7383.

https://doi.org/10.1182/blood.2023023797

THROMBOSIS AND HEMOSTASIS

Comment on Dou et al, page 1758

LNKing eosinophilia and atherothrombosis

Rainer Kaiser[1,2] and Konstantin Stark[1,2] | [1]University Hospital Ludwig Maximilian University and [2]German Centre for Cardiovascular Research

In this issue of *Blood*, Dou et al[1] reveal a decisive role for hematopoietic lymphocyte adapter protein (LNK) deficiency in promoting eosinophilia, systemic eosinophil activation, and arterial thrombus formation. The authors use pharmacological and genetic eosinophil depletion to reveal that eosinophil-specific *LNK* deficiency exacerbates arterial thrombus formation through reciprocal eosinophil-neutrophil activation and extracellular trap (ET) formation.

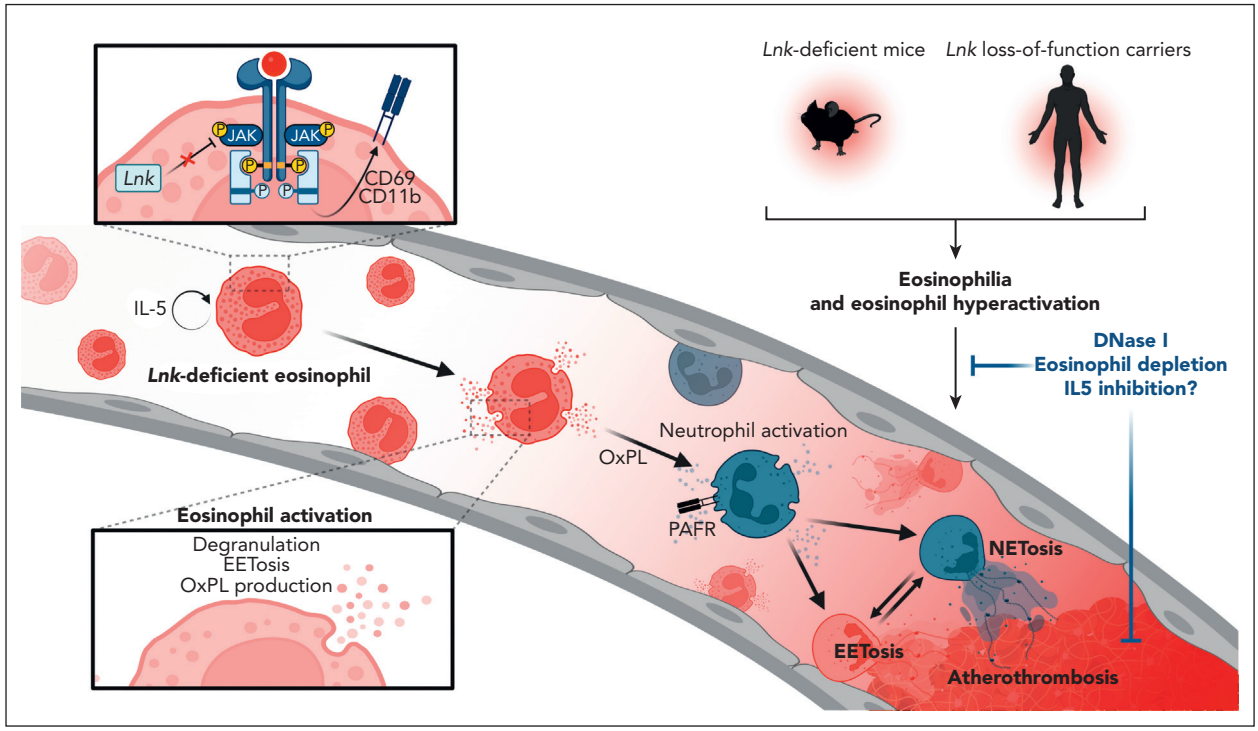
Eosinophils protect the host against parasitic infections, but eosinophilia and hyperactivation of circulating eosinophils, as observed in autoimmune diseases like eosinophilic granulomatosis with polyangiitis, are associated with collateral self-damage. This is, at least in part, driven by a prothrombotic state, including clinically relevant increases in myocardial infarction and stroke.[2] Moreover, eosinophils are enriched in arterial thrombi from patients with stroke and myocardial infarction. Recent studies have highlighted mechanistic details into how eosinophils modulate cardiovascular disease including thrombosis and associated inflammation: although eosinophils have been shown to aggravate the formation of experimental atherosclerotic lesions and arterial thrombus formation,[3-5] they also have been shown to have beneficial effects on myocardial remodeling in the setting of chronic coronary artery ligation in a mouse model of myocardial infarction.[6] The effect of eosinophils may be different in the setting of myocardial ischemia-reperfusion injury. This dichotomous role of eosinophils in cardiovascular disease warrants further investigation in a context-dependent manner.

SH2B3/LNK is a negative regulator of Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) signaling. Carriers of a single nucleotide polymorphism with functional loss of *SH2B3/LNK* have an increased risk for developing myeloproliferative disorders and are often identified due to elevated leukocyte counts with prominent eosinophilia. Importantly, carriers also suffer from an increased risk of cardiovascular disease, including myocardial infarction.[2] However, the functional link between eosinophilia in LNK-deficient individuals and the observed increases in thrombotic events are not wholly understood.

Mimicking the genetic background of T-allele carriers with LNK loss of function, Dou et al show that hematopoietic LNK deficiency increases peripheral eosinophil counts and eosinophil activation under steady-state conditions. The eosinophilia and eosinophil activation phenotypes become even more pronounced during metabolic disarray or during a chronic inflammatory state as induced by high-fat diet. Further, unleashed JAK/STAT signaling in response to interleukin-5 (IL-5) renders LNK-deficient mice more prone to forming eosinophil extracellular traps (EETs). Next, the authors used an elegant combination of genetic and pharmacological eosinophil ablation strategies, including both well-defined models such as anti-Siglec F antibody injection and ΔdblGata1 mice. To dissect the specific contribution of LNK deficiency in eosinophils to arterial thrombosis in this setting, an eoCre-Lnk$^{fl/fl}$ mouse was generated. In all of the models, eosinophil depletion consistently alleviated arterial thrombus formation in ferric chloride-induced arterial thrombosis.

An interesting finding is that in addition to increased EET formation, infiltrating neutrophils and neutrophil extracellular traps (NETs) were also frequently observed in *Lnk*-deficient mice. In line with this, the authors found that in response to eosinophil depletion, thrombus infiltration of both eosinophils and neutrophils was markedly reduced. Further, both EET and NET formation was alleviated following eosinophil depletion, pointing toward reciprocal activation loops of neutrophils and eosinophils in *Lnk*-deficient thrombi. Mechanistic in vitro experiments confirmed that *Lnk*-deficient eosinophils potently induced NET formation. On a cellular level, oxidized phospholipids (OxPL) generated by activated *Lnk*-deficient eosinophils promoted neutrophil activation in a platelet-activating factor receptor (PAFR)-dependent manner, leading to enhanced neutrophil (trans) migration and exacerbating NET formation (see figure). This observation is in line with previous work from the same authors that highlights neutrophil activation and enhanced NET formation through platelet-derived OxPL-mediated PAFR signaling.[7] However, the reciprocal communication between eosinophils and neutrophils is still not completely understood, but it is a relevant issue beyond cardiovascular diseases.

Finally, the authors used human-induced pluripotent stem cells carrying wild-type or mutated *LNK* alleles to generate eosinophils with or without functional LNK, emphasizing the translational relevance of their findings. Indeed,



Loss-of-function mutations of *SH2B3/LNK* in human carriers and LNK-deficient murine models promote eosinophilia and activate peripheral eosinophils via uncontrolled JAK/STAT signaling and downstream upregulation of surface receptors like CD11b and CD69. This activated eosinophil phenotype leads to degranulation and formation of oxidized phospholipids and promotes EET-osis. Eosinophil-released OxPLs promote neutrophil activation via PAFR-mediated signaling, leading to increased neutrophil recruitment and NET formation. Reciprocal eosinophil and neutrophil activations then culminate in a prothrombotic state, leading to atherothrombosis. Eosinophil depletion, pharmacological degradation of ETs by DNase I, or targeted approaches like inhibition of IL-5 may therefore serve as novel antithrombotic therapeutic concepts in eosinophil-driven diseases.

LNK-deficient human eosinophils were characterized by an increased activation state, as assessed by CD11b and CD69 surface expression as well as JAK/STAT signaling, and prone to form EETs in response to PAF stimulation.

The role of ETs in promoting arterial and venous thrombosis through a variety of mechanisms, including direct activation of the coagulation system, degradation of antithrombotic proteins, as well as platelet recruitment, is well known.[8,9] Conversely, soluble and platelet-derived mediators like PAF promote ET formation. The mechanism proposed by the authors—OxPL formation by hyper-activated eosinophils, subsequent neutrophil binding and PAFR-mediated activation—appears consistent with their previous work, and the in vitro experiments promote this. However, in vivo, PAFR-mediated activation may also affect platelets, which in turn may serve as a further culprit activator of neutrophils. Thus, untangling the direct or indirect effects of platelets in the interplay between eosinophils and neutrophils remains an open question.

This study emphasizes the context dependency of the intricate interplay between immune cells and coagulation and the need for specifically investigating this interplay in the right context—for example, specific genetic backgrounds and the metabolic disarray induced by a Western-type diet. It is worth noting that a previous study found no effect of eosinophil depletion on venous thrombosis in a model of inferior vena cava stenosis in wild-type mice.[5] However, given the increased risk for venous thrombus formation and thromboembolism in eosinophilia and the association with *LNK* single-nucleotide polymorphisms including the T allele,[2,10] taming eosinophils through clinically established therapeutics such as IL-5 antagonists may serve as a novel, targeted antithrombotic approach in select patients. This generates an interesting link to other settings of eosinophilic inflammation, such as asthmatic disorders or chronic obstructive pulmonary disease, which also share risk factors with cardiovascular diseases, and cases in which IL-5 inhibition is an established treatment approach and might potentially affect cardiovascular outcomes. Moreover, boosting LNK-mediated suppression of JAK/STAT signaling in genetically defined individuals at risk of thrombosis and thromboembolism could serve as an elegant precision medicine approach. However, future studies that investigate antithrombotic but also antihemostatic properties of these novel therapeutic approaches will be necessary before clinical leverage can be attempted. Further, it remains unclear whether the mechanistic insights provided by Dou et al are also applicable to patients with other disease entities driven by eosinophil hyperactivation.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Dou H, Wang R, Tavallaie M, et al. Hematopoietic and eosinophil-specific LNK(SH2B3) deficiency promotes eosinophilia and arterial thrombosis. *Blood*. 2024;143(17):1758-1772.

2. Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet*. 2009;41(3):342-347.

3. Meng Z, Zhang S, Li W, et al. Cationic proteins from eosinophils bind bone morphogenetic protein receptors promoting vascular calcification and atherogenesis. *Eur Heart J*. 2023;44(29):2763-2783.

4. Marx C, Novotny J, Salbeck D, et al. Eosinophil-platelet interactions promote atherosclerosis and stabilize thrombosis with eosinophil extracellular traps. *Blood*. 2019;134(21):1859-1872.

5. Uderhardt S, Ackermann JA, Fillep T, et al. Enzymatic lipid oxidation by eosinophils propagates coagulation, hemostasis, and thrombotic disease. *J Exp Med*. 2017;214(7):2121-2138.

6. Liu J, Yang C, Liu T, et al. Eosinophils improve cardiac function after myocardial infarction. *Nat Commun*. 2020;11(1):6396.

7. Toor IS, Ruckerl D, Mair I, et al. Eosinophil deficiency promotes aberrant repair and adverse remodeling following acute myocardial infarction. *JACC Basic Transl Sci*. 2020;5(7):665-681.

8. Dou H, Kotini A, Liu W, et al. Oxidized phospholipids promote NETosis and arterial thrombosis in LNK(SH2B3) deficiency. *Circulation*. 2021;144(24):1940-1954.

9. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A*. 2010;107(36):15880-15885.

10. Massberg S, Grahl L, von Bruehl ML, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med*. 2010;16(8):887-896.

https://doi.org/10.1182/blood.2023023530

THROMBOSIS AND HEMOSTASIS

Comment on Morelli et al, page 1773

miR-145 and incident thromboembolism

Bengt Zöller | Lund University

In this issue of *Blood*, Morelli et al[1] report that high plasma levels of miR-145 are associated with decreased risk of incident venous thromboembolism (VTE) in a population-based cohort study. The protective role of miR-145 indicates a potential novel target for VTE prevention.

Morelli et al used the Trøndelag Health Study (HUNT3), a population-based cohort, to determine whether circulating miR-145 plasma levels are associated with risk of incident VTE.[1] MicroRNAs (miRNAs), consisting of 21 to 23 nucleotides, are endogenous and noncoding small RNAs.[2] Through base pairing with target mRNAs, miRNAs regulate gene expression posttranscriptionally.[2] Circulating miRNAs refer to cell-free miRNAs in body fluids, such as plasma and serum.[2] Several studies have shown that plasma miRNAs may serve as a novel class of biomarkers in various diseases.[2] The regulatory effect of miRNA is a heritable genetic trait.[3] Several studies have linked different miRNAs to VTE but with varying results of the linked miRNAs.[2,4,5] However, the study by Morelli et al is the first larger study of a circulating mRNA as a predictor for incident VTE. Instead of using an agnostic miRNA panel, the authors used a candidate miRNA (ie, miR-145).[6-8] Patients with VTE have been found to have significantly lower plasma levels of miR-145 than controls.[6] It has been shown that administration of miR-145 mimics in a rat model of VTE resulted in a dose-dependent reduction in thrombosis formation, which was likely mediated by downregulation of tissue factor.[6] Moreover, coagulation factor XI and plasminogen activator inhibitor-1 have also been reported to be gene targets of miR-145 (see figure).[6,7] Thus, accumulating evidence supports the role of miR-145 in VTE. However, before the present study, it had not been examined whether miR-145 is associated with incident VTE.[1]

Morelli et al showed that participants in the HUNT3 study with miR-145 levels in the highest quartile had a 49% lower risk of VTE (hazard ratio [HR], 0.51; 95% confidence interval [CI], 0.38-0.68) compared with those with miR-145 in the lowest quartile in age- and sex-adjusted analysis.[1] The strongest inverse association was observed for unprovoked VTE, with an HR of 0.39 (95% CI, 0.25-0.61).[1] The results were robust and did not change after adjustment for body mass index, cancer, and arterial cardiovascular disease at the study's baseline. To consider the possibility of underestimating the true association due to regression dilution bias, the authors estimated HRs of VTE as a function of time between blood sampling and VTE. The HRs of VTE by high plasma levels of miR-145 were lower with shortened time between blood sampling and VTE events.[1] Nonetheless, the inverse relationship between miR-145 levels and VTE risk remained significant during the whole follow-up period of 7 years.[1]

Thus, Morelli and colleagues have demonstrated a robust association between miR-145 and incident VTE. What are the conclusions to be drawn from the present study? The present study indicates that miRNAs could be useful as predictors for VTE and possibly included in prediction models for VTE. The present study, together with previous human and animal studies, suggests that miR-145 overexpression might be a potential novel treatment for prevention of VTE.[1,6-8] In this context, 5q− syndrome could be mediated by miR-145 and miR-146a.[9] The 5q− syndrome is a subtype of myelodysplastic syndrome characterized by severe anemia and variable neutropenia but normal or high platelet counts with dysplastic megakaryocytes.[9] Thus, low miR-145 might lead to prothrombotic platelet changes (see figure). Another interesting issue is that miR-145 has been linked to different malignancies and arterial cardiovascular disorders (see figure).[10] This is of interest because of the linkage between VTE, cancer, and arterial vascular diseases. For example, miR-145 is downregulated in many cancers and is reported to function as a tumor suppressor.[10] In the cardiovascular system, miR-145 plays multiple roles in vascular smooth muscle cells (VSMCs), endothelial cells, fibroblasts, and cardiomyocytes (see figure).[10] For instance, VSMCs from miR-143/miR-145–deficient mice have lost their contractile abilities and favored neointimal lesion development. This suggests that these miRNAs play a role in VSMC phenotypic switch during injury and disease.[10] This is an illustration of the fact