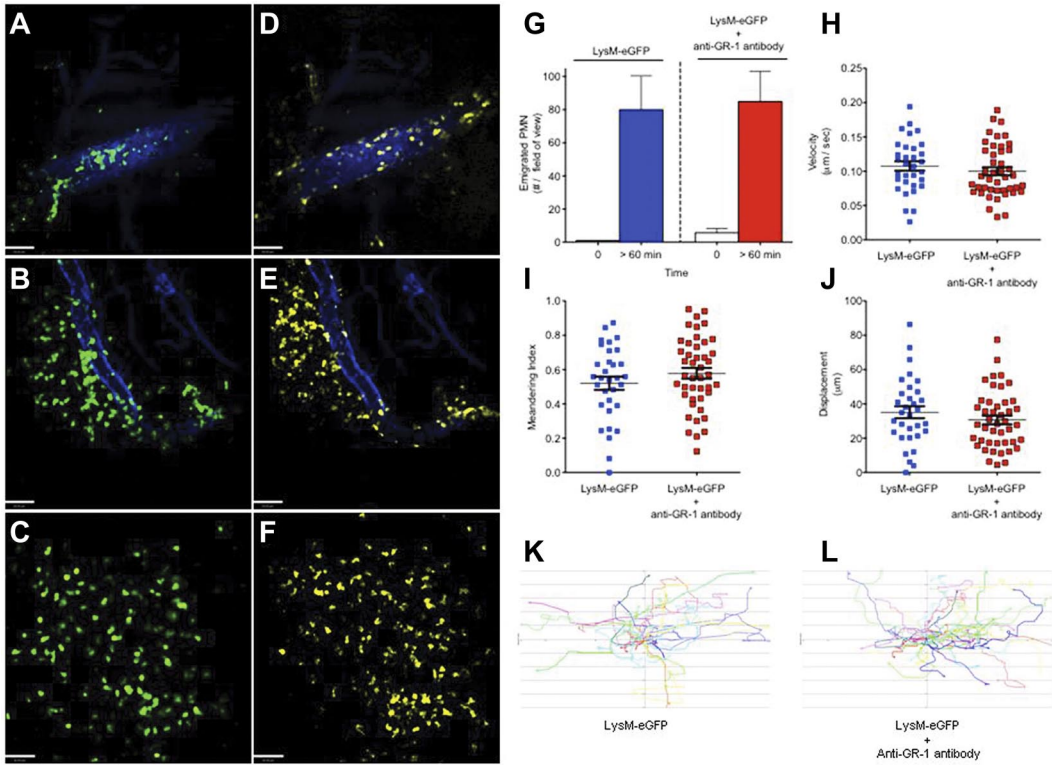

Figure 1. Direct real-time imaging of leukocyte recruitment in vivo using spinning disk confocal intravital microscopy. (A-F) *Staphylococcus aureus*–infected (intradermal 1 × 10^8 CFU of methicillin-resistant *S aureus*, USA300-2406) *LysM-eGFP* mouse skin microvasculature was imaged in vivo. Images shown in panels A through C demonstrate neutrophils defined by *LysM-eGFP* expression (green), and the corresponding side-by-side images shown in panels D through F demonstrate neutrophils defined by the anti–Gr-1 antibody (5 μg IV) conjugated to Alexa Fluor 750 (yellow) at identical time points. Endothelium was visualized using an anti–PECAM-1 antibody (10 μg IV) conjugated to Alexa Fluor 647 (blue). (A,D) Images of neutrophils rolling and adhering within a vessel captured immediately after administration of live *S aureus* into the mouse skin. (B,E) Images of neutrophils transmigrating out of a vessel captured 60 minutes after *S aureus* administration. (C,F) Images of neutrophils emigrating and chemotaxing within the parenchyma 60 minutes after *S aureus* administration. Images were captured using an Olympus BX51 upright microscope equipped with a 10×/0.3 numeric aperture air objective. The microscope was equipped with a confocal light path (WaveFx; Quorum) based on a modified CSU-10 head (Yokogawa Electric). Laser excitation at 488, 649, and 730 nm (Cobalt) was used in rapid succession with the appropriate long-pass filters (Semrock). A 512 × 512 pixel back-thinned EMCCD camera (C9100-13; Hamamatsu) was used for fluorescence detection. Velocity Acquisition Version 6.0 software (Improvision) was used to drive the confocal microscope. Images captured using the spinning disk were processed and analyzed in Volocity with linear adjustments to the black-and-white points for improved image quality. Bars represent 60 μm. Images are representative of 3 independent experiments. (G-L) Neutrophil emigration (G), parenchymal tissue crawling velocity (H), meandering index (I), displacement over 10 minutes (J), and tissue neutrophil crawling tracks (K-L) after live *S aureus* administration in *LysM-eGFP* mice without antineutrophil antibodies and *LysM-eGFP* mice that received IV anti–Gr-1 (5 μg) immediately before imaging. For cell quantification, 2 independent *LysM-eGFP* experiments were compared with 3 independent *LysM-eGFP* + anti–Gr-1 antibody experiments. All procedures performed were approved by the University of Calgary Animal Care Committee and were in accordance with the Canadian Guidelines for Animal Research.

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Contribution: B.G.Y designed and performed the experiments, analyzed the results, produced the figures, and wrote the manuscript; and P.K designed the research and wrote the manuscript.

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Response

Ly6G: a work in progress

We are grateful to Drs Yipp and Kubes for their attention to our recent work and for adding an important new piece to the puzzle of Ly6G and its function in neutrophil-mediated immunity.[1,2]

We find no incompatibility between their observations and our own. As is well recognized, the pathways mediating neutrophil migration differ with site and stimulus. In particular, whereas

LFA-1 is strictly required for neutrophil migration at the initiation phase of K/BxN arthritis,[3,4] other pathways can often compensate for β2 integrin deficiency in the context of bacterial infection.[5,6] Because we found that Ly6G ligation attenuated β2 integrin–dependent migration, but not β2 integrin–independent migration,[2] it is not clear that an effect would have been expected in the *Staphylococcus aureus* cellulitis model used by Drs Yipp and Kubes.

However, we recognize that divergent dependence on β2 integrins cannot be the whole story. The Kubes laboratory has employed conjugated anti–Gr-1 as an in vivo tool in several important studies using models of sterile inflammation in which β2 integrins play a demonstrable role.[6,7] Further investigation will be required to determine the relevant differences between these experimental systems and those used in our work.

We therefore interpret the findings of Drs Yipp and Kubes as complementary to, rather than in conflict with, our own. Together, these studies raise new and interesting questions. What are the conditions under which Ly6G-binding ligation alters migration? What is the role of Ly6G in the normal course of neutrophil physiology? What does this role of Ly6G tell us about human neutrophil biology? We look forward to working with the Kubes laboratory and others to answer these important questions.

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To the editor:

Lactate dehydrogenase and hemolysis in sickle cell disease

Lactate dehydrogenase (LDH) is one of the enzymes of the glycolytic pathway that catalyzes the conversion of pyruvate to lactate with concurrent conversion of NADH to NAD^+. It is a ubiquitous enzyme found in all tissues. Serum LDH exists in 5 separable isoenzymes numbered 1-5 according to their electrophoretic mobility.[1] The distribution of the 5 isoenzymes is not uniform across body tissues. LDH1 and LDH2 are found primarily in RBCs and heart muscle; LDH3 is highest in the lungs; LDH4 is highest in the kidneys, placenta, and pancreas; and LDH5 is highest in skeletal muscle and liver.[2] The routine determination of serum LDH includes all of its isoenzymes.

Along with reticulocyte count, indirect bilirubin level, and serum haptoglobin, LDH has been used as a marker of hemolysis. Serum LDH is usually elevated in sickle cell anemia in the steady state (SS).[3] During painful vasoocclusive crises (VOCs), the LDH may increase further in some patients because of hyperhemolysis, as shown by RBC survival studies.[4] However, the increase in LDH during VOCs is not always because of hemolysis. Neely et al found that the increase in serum LDH was not correlated with plasma Hb level, indicating that the source of LDH is not secondary to hemolysis but rather to tissue damage, most likely BM infarction.[1]

A prospective descriptive cohort study in children showed that the LDH level increases significantly during VOCs compared with steady-state values and that there is a significant positive correlation between LDH levels and the severity of pain but not between LDH and Hb.[5] Moreover, elevated LDH levels at admission for VOCs were associated with severe outcome, including death and worsening clinical state requiring transfer to the intensive care unit, in adult patients with sickle cell disease (SCD).[6]

A retrospective review of 40 patients with SS between the ages of 5 and 19 years determined correlates of microalbuminuria and proteinuria including age, sex, height, body mass index, serum creatinine, Hb level, fetal Hb, LDH level, reticulocyte count, blood pressure, history of blood transfusion, history of hydroxyurea, and history of splenectomy. The prevalence of microalbuminuria and proteinuria among the patients studied was 15% and 5%, respectively. Univariate and multivariate analyses showed a significant correlation between LDH level and microalbuminuria and proteinuria.[7] Kato et al reported an association between hemolysis and clinical subphenotypes of SCD, including pulmonary hypertension (PH), but the diagnosis of hemolysis was based on LDH levels.[8] Conversely, Ataga et al reported a significant correlation between PH and microalbuminuria, but no correlation with parameters of hemolysis.[9]