To the editor:

Shedding light on class I phosphoinositide 3-kinase activity in endothelium

Class I phosphoinositide 3-kinases (PI3Ks) play a critical role in regulating chemoattractant-induced migration of neutrophils. However, Puri et al have demonstrated that PI3K activity in vascular endothelium also contributes to the accumulation of these cells in tissues, as evidenced by the reduced ability of PI3K-deficient blood vessels to support rolling adhesion of WT neutrophils in response to the proinflammatory cytokine tissue necrosis factor α (TNFα; 20 ng). By contrast, a recent publication by Liu et al suggests that endothelial PI3K activity is not essential for this process. Although both studies used chimeric animals in which PI3K activity was present in neutrophils but absent in endothelium, it was speculated that the observed discrepancies were attributable to the amount of TNFα used to induce inflammation (500 ng vs 20 ng). We wish to point out that the use of transmitted light microscopy and electrocautery tissue dissection by Liu et al may account for these inconsistencies rather than differences in cytokine concentrations.

To confirm our hypothesis, we used WT or p110y−/− animals that were irradiated and reconstituted with WT fetal liver cells from mice expressing GFP in granulocytes, and evaluated neutrophil behavior in the microcirculation of cremaster muscle stimulated with TNFα (1000 ng). The absence of endothelial PI3Kγ activity was associated with a 5-fold increase in neutrophil rolling velocities, findings consistent with those of Puri et al (54.8 ± 4.2 μm/s vs 10.2 ± 0.6 μm/s in WT animals, P < .001, data represent means ± SEM, n = 80 cells per genotype). Moreover, the number of neutrophils extravasating into tissue was dramatically decreased in PI3Kγ chimeric animals as compared with WT counterparts (25 ± 2 cells vs 65 ± 2 cells, respectively, P < .001, Figure 1A,B,D). By contrast, the use of electrocautery rather than mechanical tissue dissection (microscissor) caused robust cell migration in PI3Kγ chimeric animals (79 ± 8 cells, P < .001, Figure 1B-D), results consistent with that of Liu et al.

Mechanistically, Puri et al speculated that class I PI3K activity in endothelium contributes to neutrophil trafficking into tissues by regulating the surface distribution but not expression of E-selectin, an adhesion molecule that mediates the rolling of these cells on the inflamed vessel wall. This is supported in the recent publication by Setiadi et al demonstrating that the physical clustering of E-selectin on cytokine-stimulated endothelium is essential for this process.5
Indeed, isoform-specific blockade of class I PI3K activity does alter E-selectin–dependent slow rolling occurring in endothelium via class I phosphoinositide 3-kinases (PI3K) when fluorescent imaging and microscissors were used for tissue dissection. By contrast, the surface distribution of PECAM-1, an adhesion molecule constitutively expressed on vascular endothelium, remained unchanged (Figure 1E,G), as did that of PSGL-1, a selectin ligand present on the surface of neutrophils (data not shown). These findings, in conjunction with the report by Sediati et al, provide a potential mechanism by which class I PI3Ks contribute to the proadhesive state of inflamed endothelium.

In conclusion, our data reaffirm the validity of the results by Puri et al and illustrate the importance of using appropriate imaging and surgical techniques when evaluating intracellular signaling pathways that play a critical role in neutrophil trafficking.

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Response

What is the appropriate surgical intervention when imaging?

We were most intrigued by the recent correspondence by Randis et al, suggesting that tumor necrosis factor α (TNFα)–induced E-selectin–dependent slow rolling occurred in endothelium via class I phosphoinositide 3-kinases (PI3K) when fluorescent imaging and microscissors were used for tissue dissection. By contrast, no role for PI3K could be seen in endothelial E-selectin function when white-light imaging and electrocautery were used as described by Liu et al. The authors directly compared the 2 techniques and concluded that the results may be related to the type of imaging and surgical approaches one uses and suggested that appropriate surgery needs to be considered when studying intracellular signaling pathways. However, what is the appropriate surgical technique for experiments? Surgical intervention however minor does induce some inflammation as was shown more than a century ago by Cohnheim when he exteriorized and imaged otherwise unperturbed frog tissues. This was subsequently confirmed by us, clearly demonstrating that even exteriorization of tissue caused some activation of mast cells and leukocyte rolling. Although Randis and colleagues caution that appropriate surgery must be used, no direct comparison to no surgery is performed, questioning which surgical approach best resembles no surgical intervention. Perhaps repeating these studies in the ear where no surgery is required would shed additional light on the issue of surgery-induced responses. Similarly, fluorescence imaging compared with white light has been suggested to perturb the microcirculation. Whether white light can also alter the microcirculation is difficult to test, as we have nothing to compare it to.

The mechanism of action by which the 2 surgical approaches differ may be related to either the activation of an additional PI3K pathway with microscissor surgery in endothelium or the turning off of a PI3K pathway using electrocautery. In both situations the 2 groups provide control preparations that appeared not to be activated. It is therefore likely that the surgical interventions must impact pathways activated by TNFα. Electrotaxis has recently been shown to be a real physiologic property of immune cells that is dependent upon PI3K which could explain some of the results but this remains to be elucidated. We would, however, wholeheartedly agree with the very important message of Randis et al, reminding us that anytime an animal is anesthetized, surgically manipulated (by whatever means), and exposed to fluorescence or white light, there is always the potential for the modification of signaling pathways involved in basal physiology and induced pathology.

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References

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