by IL-33 is similar to that provoked by IL-3, the most potent basophil activator. However, the signaling pathways activated by IL-33 in basophils are different from those triggered by IL-3. Whereas IL-3 primarily leads to the activation of the JAK/STAT pathway and ERK in basophils, IL-33 primarily activates the NFkB pathway and the p38 MAP kinase. In line with this observation, IL-33 did not mimic all effects of IL-3 on basophils. Likewise, in contrast to IL-3, IL-33 did not prime human basophils for C5a-induced LTC4 generation.

Other studies have recently confirmed that IL-33 is a regulator of human basophils. One interesting effect of IL-33 on basophils is induction of CD11b, suggesting that IL-33 may also regulate basophil adhesiveness to endothelial cells (which can produce IL-33) and thus can enhance basophil-transmigration from blood into tissues. In addition, IL-33 may trigger migratory responses of human basophils.

An unexpected finding in the study by Pecaric-Petkovic and colleagues was that basophils apparently express only trace amounts of IL-33 receptor T1/ST2 on their surface. In fact, in resting basophils, the receptor was only detectable by polymerase chain reaction, not by flow cytometry. Nevertheless, the effects of IL-33 on basophils could be blocked by an antibody against ST2, suggesting a functional interaction on resting basophils. So far, little is known about the regulation of expression of the IL-33 receptor (ST2) on basophils or other immune cells. When exposed to IL-3, basophils were found to up-regulate and thus display considerable amounts of surface ST2 protein. These data suggest that IL-3 can regulate IL-33 responsiveness of basophils, and thus confirm that IL-3 is a major basophil regulator. In addition, this observation predicted cooperative effects of IL-3 and IL-33 on basophils. Indeed, IL-3 and IL-33 were found to cooperate with each other in promoting cytokine (IL-4) secretion in human basophils.

Basophils also express receptors for IL-1 and IL-18. However, in contrast to IL-3, neither IL-1 nor IL-18 were reported to exert major proinflammatory effects on human basophils, which is of interest since mouse basophils are responsive to IL-18. On the other hand, it is well known that mouse basophils differ from human basophils in many aspects, and the same holds true for mouse and human mast cells.

Apart from basophils, eosinophils and mast cells also appear to be IL-33 targets. With regard to mast cells, IL-33 apparently also promotes adhesion as well as secretion of mediators and cytokines. Similarly, it has been described that IL-33 triggers eosinophil activation, expression of CD11b, and adhesion. An interesting aspect is that IL-33 activates p38 in human blood eosinophils in the same way as in basophils, whereas other blood leukocytes are not activated by IL-33. All in all, it seems as if IL-33 is a major regulator and activator of (the secretory function of) immune cells that play a role in acute inflammation. The same regulator may also trigger the rapid recruitment of these cells into tissue sites (of inflammation) through increased adhesiveness and migration induction. In addition, it has been shown that IL-33 promotes the survival of eosinophils and mast cells in vitro. Whether IL-33 can also promote basophil survival and differentiation of these cells (basophils, eosinophils) from their multipotent uncommitted progenitor cells, as has been described for IL-3, remains at present unknown.

In summary, IL-33 is an emerging regulator of certain immune cells involved in allergic and other immediate inflammatory reactions, and thus may be a key cytokine in the pathogenesis of diseases in which activation of basophils, mast cells, and eosinophils plays an essential role, such as allergic or chronic inflammatory diseases. These observations may also provide the basis for the development of new anti-inflammatory drugs that target the expression, release, or function of IL-33 or IL-33 receptors.

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Comment on Huang et al, page 1589

Fishing for platelets

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Platelets can be recruited by an ultra-large multimer fraction of von Willebrand factor extending from the endothelial surface into the plasma flow as long strings. In this issue of Blood, Huang and colleagues investigate the complex structure of these fishing lines and uncover a role for ανβ3 integrin as their anchor to the endothelial surface.

Immediately after activation, endothelial cells release long strings of highly-multimerized von Willebrand factor (VWF) that recruit platelets to form “beads on a string” (see figure). Previously documented both in vitro and in vivo, these strings described a dramatic new mechanism by which platelets can be recruited. However, not much is known about the strings. Both structural and functional questions of fundamental importance are still unanswered, although their structure does explain the need to store VWF.
coiled into tubules within Weibel-Palade bodies (an orderly unfurling is required to effectively cast a fishing line), neatly explaining the unique rod-like shape of this organelle.

How are the strings anchored to the endothelial surface? Huang et al provide a new set of data showing that αvβ3 integrins play an important role, in particular by using lentivirus-expressed shRNA-mediated knockdown of αvβ3 in human umbilical vein endothelial cells. Anchorage is important because stretching of VWF under flow exposes the cleavage sites used by the TTP-associated protease ADAMTS13. Since this cleavage is needed to cut the dangerously prothrombotic ultra-large VWF into safer fragments, and stretching requires a firm anchor, this is of real importance. However, conclusive identification of string-anchoring molecules has proven difficult. Targeting of P-selectin to WPB by its binding of VWF plus blockage of in vitro string formation by anti–P-selectin antibodies or soluble P-selectin suggest a role for this receptor as an anchor. However, intravital microscopy within mesenteric venules shows that mice deficient in P-selectin can still make strings. A role in string-anchoring for αvβ3 was also ruled out by Padilla et al in vitro, and by Andre et al and Chauhan et al in vivo.

What underlies these differences? Perhaps at the lower shear stress found within venules, adhesion by integrins and by P-selectin may be less important than at the higher shear stress occurring in arterioles that was used in the in vitro experiments. This emphasizes differences in string behavior between types of endothelia. In addition, variability between the experimental conditions that have been used in binding studies are likely involved.

Less controversially, all studies suggest a small number of anchorage points per cell, and Huang et al suggest that changes in direction of flow can lead to formation of new attachment sites. Light microscopy from Huang et al shows αvβ3 in patches along the strings. Whether these are all tethering points is not yet clear.

How are such long strings made? Since the very largest VWF multimers are the most efficient at recruiting platelets, size definitely matters. The simplest question here is whether a single multimer is equivalent to 1 string. This was unlikely since strings can be up to several millimeters long, whereas structural studies suggested that a multimer coiled into a tubule running the length of a very large Weibel-Palade body (5 μm) could extend to about 250 μm. Self-association of VWF has been previously described as well as the formation of multistranded bundles but in their paper, Huang et al use high-resolution scanning electron microscopy of acutely-secreted VWF to reveal a variety of forms of self-associated molecules, forming twisted bundles and networks that likely account for the observed length of the strings.

Finally, one entirely unexpected finding by Huang et al is that a significant subset of strings carry no platelets at all. The only real clue as to why this might be is that an increase in flow does slightly increase the fraction of platelet-bearing strings, suggesting that the structure of strings changes to reveal interior platelet-binding sites, but whether changes in turbulence and shear within the vasculature are sufficient to raise the fraction to one, or whether some strings just have a different function remains unanswered for now.

How important is all this? The VWF released from endothelial cells in immediate response to secretagogue-stimulation is the most prothrombotic, spontaneously platelet-binding form of this molecule, implying that it must play an important role in recruitment of platelets. However, the strings seem to be short-lived, disappearing within minutes. Despite this, and assuming a simple relationship between the ultra-large VWF seen on multimer gels and VWF in strings, the hemostatic significance of ultra-large multimers as shown by von Willebrand disease mutations that reduce this fraction of VWF suggests that ultra-large VWF is indeed important. Just how this “μfishing” is carried out is therefore of high interest.

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

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