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Oxidative Modification of Leukocyte Adhesion

In this issue of *Immunity*, Marko Salmi and colleagues describe mice lacking AOC3, an endothelial cell monoaminooxidase that is involved in modulating leukocyte rolling, adhesion, and migration (Stolen et al., 2005). Their data demonstrate the importance of oxidative modification of (unknown) adhesion molecules in regulating inflammation and lymphocyte homing.

Amino oxidase, copper-containing-3 (AOC3), also known as vascular adhesion molecule-1 (VAP-1), is an ectoenzyme that is constitutively expressed in adipocytes and endothelial and smooth muscle cells of most organs of mice and humans (Jalkanen and Salmi, 2001). Activity is also found in blood serum, suggesting that the enzyme may be shed from the endothelial surface. AOC3 catalyzes oxidative deamination and produces aldehyde groups, hydrogen peroxide, and ammonium. Although enzymatic modifications of adhesion molecules are common, modification by an amino oxidase has not been described. Using gene targeting and homologous recombination, the authors have produced mice that lack detectable AOC3 activity from all organs tested (Stolen et al., 2005). Although these mice are viable and healthy under vivarium conditions, they show interesting defects in lymphocyte homing and in the inflammatory response to cytokine or autoimmune challenges. In short-term homing assays, $AOC3^{-/-}$ mice show reduced lymphocyte homing to mesenteric lymph nodes (MLN) and spleen, which also show slightly reduced cellularity.

What exactly does AOC3 do? As originally proposed, AOC3 may directly serve as an adhesion molecule, binding an unknown ligand (Salmi and Jalkanen, 1992). However, there are no data supporting such a direct adhesive interaction, which would typically be shown in a cell-free reconstitution assay. In fact, circumstantial evidence presented in Stolen et al. (2005) suggests that it is indeed the enzymatic activity of AOC3 that matters for its function. First, all AOC3 antibodies with antiadhesive properties also block its enzymatic activity. Second, if an adhesion molecule is blocked by immunoneutralization, the effect is observed immediately, but injecting AOC3 antibodies into mice raises leukocyte rolling velocity over a 30 min period (Figure 4A in Stolen et al. [2005]), consistent with a requirement for protein turnover that might remove an enzymatic modification. Indeed, the data suggest that selectin ligands may be targets of enzymatic regulation. Neutrophil rolling velocity is increased 5- to 6-fold in a model of cytokine-induced inflammation in venules of the cremaster muscle (Stolen et al., 2005). This is reminiscent of the phenotype seen in E-selectin-deficient mice (Kunkel and Ley, 1996).

E-selectin engagement not only modulates leukocyte rolling velocity, but also activates rolling neutrophils (Simon et al., 2000) and results in their adhesion on inflamed endothelial cells. This activation pathway is redundant with chemokine-dependent neutrophil activation, such that neutralizing chemokine receptor signaling or removing the chemokine receptor CXCR2 in mice has no effect on neutrophil recruitment unless E-selectin is also blocked (Smith et al., 2004). E-selectin engages an unknown ligand to activate rolling neutrophils that is different from L-selectin or P-selectin Glycoprotein Ligand-1 (Smith et al., 2004). Conceivably, absence of AOC3 impairs the neutrophil-activating capacity of this interaction, which might explain the defect in neutrophil adhesion seen after 3 or 6 hr of TNF-α (Stolen et al., 2005). Slow neutrophil rolling and subsequent adhesion in inflammation requires not only E-selectin but also β_2 integrins (CD18) (Jung et al., 1998). β_2 integrin-dependent slow neutrophil rolling is prominent when these integrins are stabilized in a partially activated conformation (Salas et al., 2004) or when their I-domain is expressed in isola-

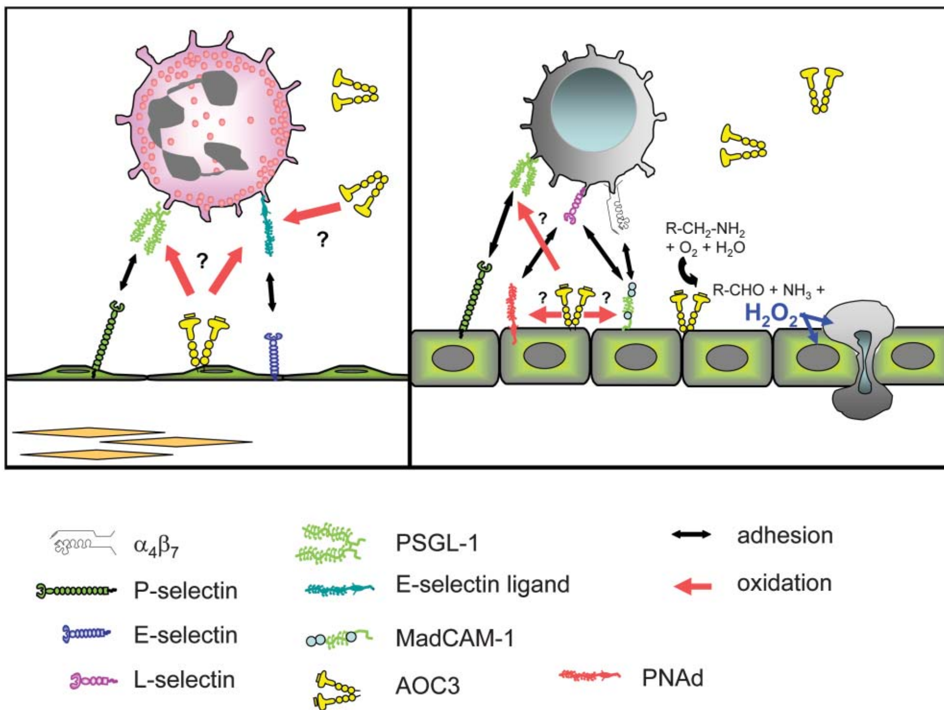

Figure 1. AOC3 Regulation of Leukocyte Migration May Be Dependent upon Modulation of Adhesion Molecules and/or Cell Signaling

AOC3 expressed on endothelial (green) and smooth muscle cells (not shown) and circulating in the blood may modify adhesion molecules on the endothelial as well as the leukocyte surface to modulate leukocyte trafficking. Based on the data presented in Stolen et al. (2005), candidate adhesion molecules include E-selectin ligands on neutrophils (left) and L-selectin ligands on high endothelial cells (right). In addition, byproducts generated by AOC3 enzymatic activity such as H_2O_2 may regulate endothelial and/or leukocyte cell signals necessary for transmigration, including MMP activation, protein tyrosine phosphatase inhibition, and/or gene expression.

tion (Knorr and Dustin, 1997). This means that there is ample room for AOC3-dependent modifications of neutrophil rolling and adhesion beyond just modifying E-selectin ligands. Some possibilities are shown in Figure 1.

Lymphocyte homing to peripheral lymph nodes and Peyer's patches is largely L-selectin dependent (von Andrian and McKay, 2000). L-selectin ligands in secondary lymphoid organs are fucosylated, sialylated, and sulfated carbohydrates that are collectively known as PNAd, or Peripheral Node Addressins. Interestingly, mild oxidation of PNAd such as might be expected from the action of AOC3 has been described to produce a Schiff base and thus stabilize L-selectin interactions (Puri and Springer, 1996). The data reported in Stolen et al. (2005) are consistent with L-selectin ligands being modified by AOC3 activity, which may increase their binding to L-selectin. However, L-selectin is not involved in lymphocyte homing to the spleen, which is also reduced in AOC3$^{-/-}$ mice. This suggests that AOC3 may have other unknown effects.

Interestingly, Stolen et al. (2005) found an even stronger 71% reduction in neutrophil transmigration compared to a 50% reduction in firm adhesion, suggesting that an AOC3-dependent modification or interaction primes neutrophils for transendothelial migration. Potentially, H_2O_2 generated by AOC3 enzymatic activity may modulate lymphocyte migration. Salmi et al. (2001) demonstrated that exogenous addition of H_2O_2 to VAP-1-expressing rabbit cardiac endothelium does not enhance lymphocyte rolling and adhesion. However, transmigration was not examined. AOC3 is only one among many sources of H_2O_2. During VCAM-1-dependent lymphocyte migration, VCAM-1 signaling in endothelial cells activates NADPH oxidase, a plasma membrane enzyme complex that generates low levels of extracellular ROS that have intracellular and cell surface effects. Inhibiting NADPH oxidase expression or scavenging ROS blocks VCAM-1-dependent lymphocyte migration (Matheny et al., 2000). In analogy to these observations, AOC3-generated H_2O_2 may also regulate lymphocyte migration.

Even though the current paper does not provide mechanistic insight into how AOC3/VAP-1 really works, it unequivocally shows that this enzyme modulates neutrophil and lymphocyte trafficking. The authors show reduced disease incidence and lethality in a model of autoimmune diabetes, reduced leukocyte infiltration in a model of peritonitis, but unchanged lethality in response to *Yersinia* infection. Because the inflammatory and immune defects are mild in AOC3$^{-/-}$ mice, it is possible that blocking this enzyme could have useful anti-inflammatory effects. Whether or not therapeutic trials are successful, the availability of AOC3$^{-/-}$ mice opens the door for conducting investigations into the molecular mechanisms of AOC3 action in leukocyte trafficking.

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