Conduit for Privileged Communications in the Lymph Node

A study by Sixt et al. in this issue of Immunity identifies conduit-associated dendritic cells whose privileged access to antigen arriving by the conduit ensures the initiation of T cell activation within a few hours of antigen exposure rather than a day later after the arrival of dendritic cells from skin.

The cortex of lymph nodes is like a railway grand concourse through which traveling T- and B-lymphocytes encounter antigen presented by dendritic cells and respond by becoming activated or by dividing to produce daughter effector populations. Lymph node function depends on an extraordinary architectural scaffolding that solves daunting logistical challenges, in part by transmitting soluble information through the very same reticular fibers that provide essential structural support for the blood and lymph vasculature that are the portals for hematopoietic cell entry and exit (Anderson and Shaw, 1993). The lymphocytes and mononuclear cells making up the bulk of lymph node occupants are transient visitors that arrive and move through labyrinthine corridors and along reticular cell escalators to compartments where they may interact with specific antigen presenting cells, depending on their phenotype and functional role in immune responses. In this issue of Immunity, Michael Sixt and his colleagues reveal that the fibroblastic reticular cell conduit (FRCC) telegraphs privileged communications across the crowded lymph node cortex to dendritic cells (DC), adding a new function to the known role of the FRCC in transmitting signals to the high endothelial venule (HEV) (Gretz et al., 2000).

Sixt and colleagues identify a subset of dendritic cells that has three cardinal properties: these DC are in direct contact with the conduit, they appear to take up antigenic-tracer from the conduit, and they can process the antigen thus acquired. Sixt and colleagues refer to these DC variously as “conduit associated,” “fiber associated,” or “resident.” For use herein, we choose their term “conduit-associated” DC as the most useful functional description. They distinguish those DC from other DC present nearby that are not in direct contact with the conduit and do not rapidly acquire/process antigen present in the conduit. These findings confirm and extend previous demonstrations of uptake/presentation of antigen by lymph node DC at early time points after immunization. For example, Itano and colleagues identified populations of DC in lymph nodes that mediate rapid uptake of antigen (peak 2 hr) (Itano et al., 2003). Prior to such findings, a prevailing view was that the relevant DC were ones that brought antigen from the site of immunization to lymph node cortex, a process that takes about 24 hr. The compelling appeal of the conduit-facilitated antigen delivery to lymph node DC is that it ensures the initiation of T cell activation within a few hours of antigen exposure rather than a day later after the arrival of migrating DC.

One particularly interesting issue that is raised in this paper is how the conduit-associated DC is positioned to acquire antigen from the FRCC. Sixt and colleagues used immunofluorescence to characterize the extracellular matrix components and integrins that FRCC use to lie upon and invest the FRCC. Fluorescent-labeled ER-TR7, which allows one to see the FRCC cytoplasm and an extracellular component, and fluorescent antibody to perlecan, which allows one to see basement membrane, were used in combination with soluble fluorescent tracer to show that DC membranes interrupted the FRCC cytoplasm and, thus, were in direct contact with the conduit conducting the soluble antigen. These phenomena are easily visualized in Figure 1B but is typically not well seen on EM and may merge with the interior surface of the basement membrane that surrounds it; (3) basement membrane, in which laminins 8 and 10, perlecan, and type IV collagen are highly abundant; it is thus similar to endothelial basement membrane and thus capable of providing a sufficient seal to prevent leakage on its own; (4) and fibroblastic reticular cells whose bodies form an additional circumferential seal. Given this specialized seal around the conduit core, how can fluid-borne material exit? Hayakawa and colleagues demonstrated that macrophages and DC tightly contact 10% of the conduit surface area (Hayakawa et al., 1988). Our own studies confirm such interactions and indicate that specialized contact regions (Figure 1C) can enable direct access of the conduit-associated DC to fluid in the conduit core (Gretz et al., 1996, 2000; Kaldjian et al., 2001). These associated DC may be lost during specimen processing, thereby giving rise to apparent “gaps” in the conduit covering. By occupying and sealing the “gap” in the FRCC, these DC receive, process, and present soluble antigens without leaking antigen to all other lymph node occupants.

A second issue that is certain to be complex and controversial is the origin and phenotype of the relevant DC. Conduit-associated DC are likely to correspond to morphologically defined “interdigitating dendritic cells” (IDC) which are found in T cell cortex and typically associated with conduits. However, IDC are themselves complex, consisting of at least four different phenotypes in tonsil (Summers et al., 2001) and presumably differing in origin. The phenotypic data on DC in lymph node that become antigen positive within a few hours of antigen inoculation is already somewhat contradictory. Sixt and colleagues phenotype these cells by fluorescence mi-
Figure 1. Transmission Electron Micrographs of Views of a Conduit

(A) The conduit is cut across the fiber revealing individual collagen fibers (asterisk) as spots of two sizes; the larger are type I collagen, and the smaller are type III collagen. Collagen fibers are separated from each other by space held open by cross bridges of keratan sulfate and possibly other ECM proteoglycans. The gray amorphous material (f) along the perimeter of the collagen core corresponds to both the basement membrane and fibrillin components, because they cannot be distinguished in cross-sections. The entire conduit is surrounded by a fibroblastic reticular cell (FRC); a junctional complex is visible at the left where the two sides of the FRC meet, thereby providing a cell-based water tight seal that augments the seal provided by basement membrane.

(B) The conduit is cut longitudinally just inside of the perimeter of the fiber. This grazing cut exaggerates the fibrillin layer (f) that can be seen on either side of the collagen fibers (asterisk).

(C) Cross cut of a conduit with large collagen core (asterisk) supporting a dendritic cell (DC). In the upper left, around ten o’clock, a process of the DC (arrow) can be seen inserting itself between the separated edges of the two sides of the FRC process (FRC). The preparation is from an adjuvant inoculation study where significant numbers of dendritic cells entered the lymph node from the blood and inoculation site, and, therefore, presumably this is a “differentiating” dendritic cell that is initiating a firm connection to the conduit.
In this issue of Immunity, Marko Salmi and colleagues describe mice lacking AOC3, an endothelial cell mono- 
amino oxidase 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 ectoen- 
zyme 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 cata-
lizes 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, bind-
ing an unknown ligand (Salmi and Jalkanen, 1992). How-
ever, there are no data supporting such a direct adhesive interaction, which would typically be shown in a cell-
free reconstitution assay. In fact, circumstantial evi-
dence 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 in-
creased 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 activa-
tion, such that neutralizing chemokine receptor signal-
ing 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 Gly-
protein Ligand-1 (Smith et al., 2004). Conceivably, absence of AOC3 impairs the neutrophil-activating ca-
pacity 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 adhe-
sion in inflammation requires not only E-selectin but also β2 integrins (CD18) (Jung et al., 1998). β2 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-