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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 enables uptake and processing of antigen within 90 min of antigen inoculation, long before 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 FRC use to lie upon and invest the FRCC. Fluorescent-labeled ER-TR7, which allows one to see the FRC 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 FRC cytoplasm and, thus, were in direct contact with the conduit conducting the soluble antigen. This clearly demonstrated that some DC were positioned to be able to sample and present antigenic conduit contents. Figure 1 provides electron-microscopic images that complement their data. The structure of the conduit can be viewed as a four-layer structure composed (from the center out) of: (1) a core of type I and III collagen bundles; the void volume between bundles is the space in which chemokine and soluble antigen is transported; (2) a microfibrillar zone that is composed in large part of fibrillins; this layer is unusually well 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 FRC, 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-

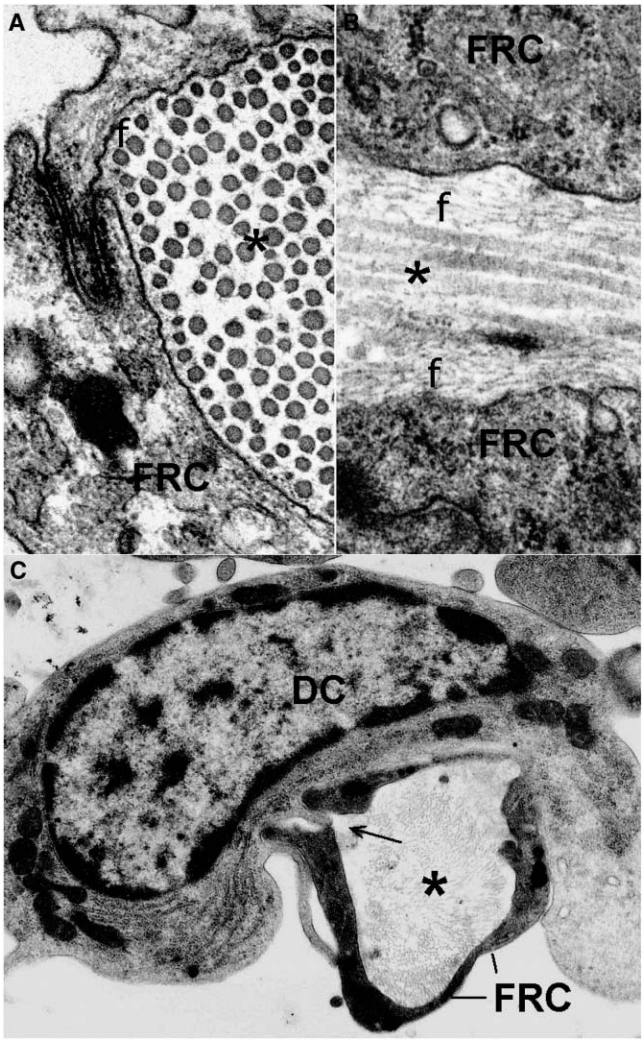

Figure 1. Transmission Electron Micrographs of Views of a Conduit

(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.

croscopy as CD11b+ CD11c+, DEC205− and conclude that these are myeloid dendritic cells that presumably had previously entered lymph node from the blood. To further characterize differential adhesion capacities of such cells, they study in vitro bone marrow-derived dendritic cells. In contrast, Jenkins and colleagues determine that the dendritic cells that become antigen positive by 2 hr after subcutaneous inoculation are MHC class II^{hi} and conclude that such DC originate from the skin (Ingulli et al., 2002; Itano et al., 2003). Given technical differences (phenotyping by flow cytometry of recovered cells versus immunofluorescence analysis of cells in situ) and experimental differences (e.g., footpad versus ear inoculation), firm conclusions on the origins of such conduit-associated DC must await further investigation.

Another recent report is pertinent to discussion of how lymph node architecture facilitates antigen presentation by dendritic cells. Using adoptive transfer of fluorescent transgenic cells, Bajenoff and coworkers described preferential localization of tissue-derived dendritic cells near HEV (Bajenoff et al., 2003). They demonstrated that resulting T cell activation clusters are enriched in the peri-HEV region populated by such dendritic cells. Recently, in vitro binding assays on lymph node frozen sections were used by Katakai and colleagues to show that a T cell area of lymph node cortex that is rich in FRCC exhibited preferential binding of DC. They named this area the "cortical ridge" (Katakai et al., 2004).

Thus, the immune system provides at least a dual strategy for antigen processing and presentation by DC in the lymph node; both strategies are strongly facilitated by strategic anatomic organization. A very early wave of antigen is delivered to conduit-associated DC that initiates T cell stimulation resulting in both T cell activation (reflected in CD69 expression) and cytokine secretion (for example, IL-2). DC from skin arrive about 24 hr later bearing much higher concentrations of antigen; they efficiently position themselves near the HEV to maximize contact with incoming T cells. The stimulus provided by such migratory dendritic cells provides additional activation that results in sustained IL-2R expression and efficient promotion of delayed type hypersensitivity (Itano et al., 2003). The dynamics of T cell/dendritic cell interactions in vivo are now the subject of intense investigation.

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