

A novel immune processing mechanism in the Leydig organ of the skate, *Leucoraja erinacea*

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Elasmobranchs, the taxonomically oldest gnathostomes, are known to be the first animals to possess adaptive immunity. While multiple theories exist to explain what spurred the development of this specific and robust arm of the immune system, definitive evidence remains elusive. In this study, we use histological analysis to describe a novel immune processing mechanism, which we refer to as *pathogen trapping*, within a unique hematopoietic organ in the esophagus of elasmobranchs, the Leydig organ (LO). We believe this development may shed light on the evolution of antigen presenting mechanisms by the immune system.

The Leydig organ (LO) is a leukopoietic tissue unique to elasmobranchs located between the mucosa and muscularis of the esophagus.⁸ Since ultrastructural analyses in the 1980s, which employed transmission electron microscopy to characterize the various leukocytes produced by the tissue, the LO has gone relatively unresearched.^{4,6} The most recent studies of the LO were conducted a decade or more ago, and focused primarily on expression of genes associated with lymphocyte evolution and development.^{1,2,7,10} The aim of this study was to histologically investigate the cells and vascular architecture of the LO for an ongoing project to assess stem cell activity in *L. erinacea*. Leydig organs were processed for paraffin sectioning and hematoxylin (H) and eosin (E) staining. During our analysis, we discovered what appears to be an immune processing pathway we refer to as “pathogen trapping”, an analogy to “antigen trapping”, which has been used to describe phagocytosis and pinocytosis.¹² Once identified, we set out to understand the mechanism of pathogen trapping in order to augment our understanding of this leukopoietic microenvironment within *L. erinacea*.

In our histological characterization of the LO, we observed a parasitic nematode based on images and descriptions from other studies describing parasitic nematodes.¹¹ We observed the nematode being engulfed by a hollow compartment formed by aggregation of squamous epithelial cells from the inner lining of the esophagus (Fig 1A,C). Epithelial cells arranged themselves into cuboidal shapes in small groups and formed the walls of hollow tubules. Formation of the tubules is thought to be triggered by pathogenic contact.

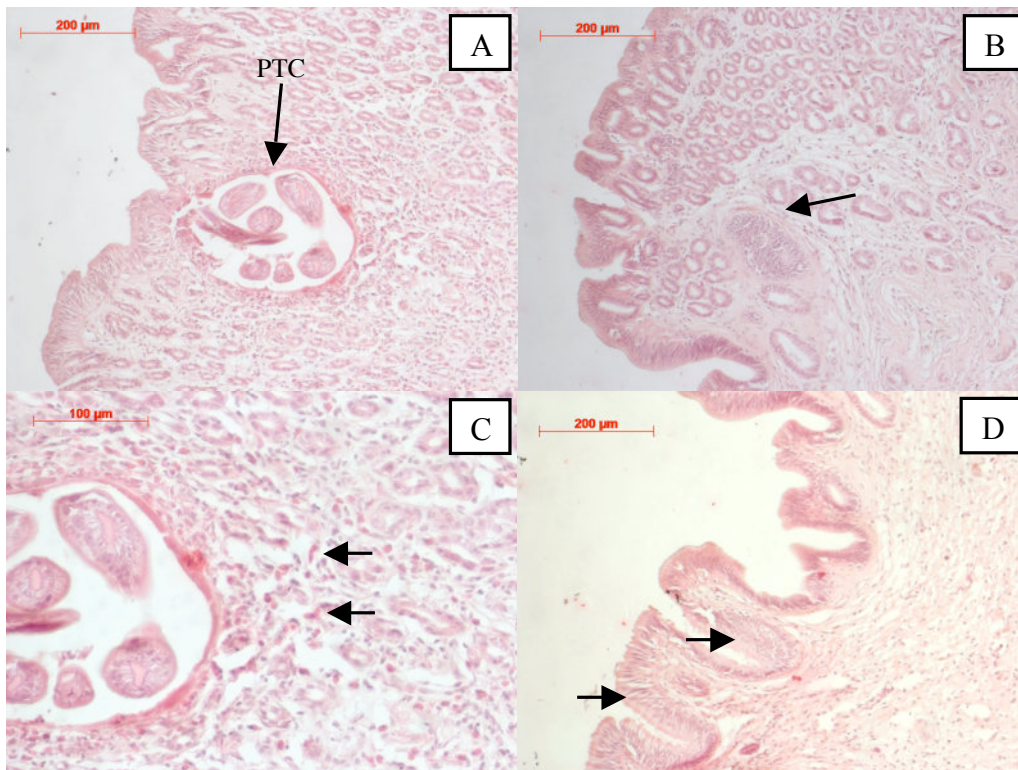


Figure 1. H and E stained pathogen trapping in the Leydig organ. A, Transverse section through tubules in the inner lining of the esophagus; epithelial cells coalesce forming a pathogen trapping compartment (PTC) to envelop the nematode and carry it to the LO, located out of frame to right. B, Clusters of epithelial progenitors (arrow) forming to regenerate the epithelium. C, Eosinophils (arrows) migrate to PTC to encounter the pathogen. D, Epithelial progenitors (arrow) proliferate along side a fingerlike projection to restore depleted epithelium.

While epithelial cells form many tubules, only the epithelial cells in direct contact with the pathogen are able to engulf it and form a pathogen trapping compartment (PTC). We observed the tubules to be approximately 25-50 μm in diameter with the exception of the PTC. This was significantly larger ($\sim 250 \mu\text{m}$), yet comprised of a similar number of cells. This PTC appeared to be detaching from the epithelial lining and migrating through the lamina propria into the LO (Fig 1A,B). Large quantities of eosinophilic granulocytes were also observed moving from the LO toward the PTC (Fig 1C). We believe degranulation of these cells assists in pathogen destruction, although further analysis is warranted. In serial transverse sections of the LO, we also observed clusters of small, densely nucleated cells located near the epithelium, alongside villi of the epithelium, associated directly with the nematode (Fig 1D). These cell clusters were found where epithelium appeared to be depleted due to production of tubules. Therefore, we believe they are epithelial progenitor cells regenerating the epithelial lining; again, more comprehensive analysis will be required to verify such a claim.

In summary, we hypothesize that the observed pathogen trapping may represent a novel immune mechanism related to antigen presentation in mammalian lymph nodes. Intuitively, it would be reasonable for innate immune sentinel cells to engulf, digest, and dispose of the pathogen. Given the lack of lymphatics in these species, destroyed pathogens would be eliminated via the gastrointestinal tract.³ Instead, we propose the degrading pathogen is transported to the LO for antigen presentation to lymphocytes.^{1,6} While the LO has been historically considered a primary immune organ, this indicates it may have secondary immune function. This may represent a precursor mechanism to the lymphatic system, in which antigen-presenting cells are transported to lymph nodes containing the adaptive immune cells required for more robust and long-lived immune responses. Although lymphatics are mostly absent in elasmobranchs, small venous vessels have accessible inter-endothelial junctions and other structures very similar to those of the initial lymphatics.³ These observations also support the hypothesis that when jaws first developed in vertebrates (in the elasmobranchs) and new food sources became available, these species were exposed to a wide variety of new, possibly more complex pathogens.⁸ The location of the LO within the esophageal wall would be the first anatomical structure encountered by these pathogens; thus, elasmobranchs may have co-evolved the adaptive immune system to combat these novel pathogens.⁵

This work was supported by an Institutional Development Award (IDeA; NIH-NIGMS) P20GM103423. We thank Dr. James Sulikowski of the University of New England for providing the animals used in this study.

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