

INSIGHTS

Hiding in plain sight: A new lymphoid organ discovered in zebrafish

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A small, unassuming, fleshy lobe at the base of the pectoral fin of fish has long been overlooked by scientists. In this issue of *JEM*, Castranova et al. (<https://doi.org/10.1084/jem.20241435>) describe this structure as a new secondary lymphoid organ. Translucent and externally located, the axillary lymphoid organ (ALO) shares striking structural similarities with mammalian secondary lymphoid organs. Due to its position and optical accessibility, the zebrafish ALO permitted noninvasive, high-resolution imaging of immune cell dynamics in live animals. These studies revealed likely interactions between T, B, and macrophage cells, arguing that the ALO may function in adaptive immune cell activation and provide a nexus for immune cell trafficking and communication.

The vertebrate immune system is comprised of a complex network of cells, tissues, and organs that all coordinate to defend against pathogens. It is made up of two interconnected components: the innate and adaptive immune systems. The innate immune system, which includes macrophages, reacts quickly to infecting pathogens through germline-encoded pattern recognition receptors with no need for prior pathogen exposure. By contrast, the adaptive immune system is characterized by its immunological memory and the capacity for generating antigen-specific responses (Melo-Silva and Sigal, 2024). Adaptive immune response requires the exquisite orchestration of antigen presentation to helper T cells, followed by T cell interaction with B cells to induce their differentiation into plasma cells and ultimately culminates in the production of antigen-specific antibodies by B cells. Interactions between antigen-presenting cells and lymphocytes are the foundation of robust immune responses and largely happen in the secondary lymphoid organs, including the lymph nodes, spleen, and mucosa-associated lymphoid tissues (MALTs) (tonsils, Peyer's patches in the intestine, and bronchus-associated lymphoid tissue).

The thymus is the primary organ for T cell development in teleost fishes, including zebrafish (*Danio rerio*), while the adult anterior kidney marrow is the likely site of B cell generation and red blood cell production (Björge and Koppang, 2021). Teleost fishes lack lymph nodes and do not contain Peyer's patches in their intestine, raising questions about how adaptive immune surveillance functions in these organisms. The spleen is widely considered to be the dominant secondary lymphoid organ in teleost fish. Indeed, Shibasaki and colleagues recently uncovered unique lymphoid structures in the trout spleen where antigen-presenting cells and T and B cells interact (Shibasaki et al., 2023). These melanomacrophage centers associated lymphoid aggregates contain large numbers of proliferating CD4⁺ T cells juxtaposed with IgM⁺ B cells that are undergoing somatic hypermutation. Hu et al. also recently suggested the dual role of the anterior kidney as both a primary and secondary lymphoid organ in zebrafish (Hu et al., 2024). Viral exposure induced the proliferation of CD4⁺ T, CD8⁺ T, and IgM⁺ B cells in the fish kidney marrow, suggesting immune responses can also be initiated in this organ. Robertson et al. also recently uncovered a network of T lymphocytes that are hexagonally arranged



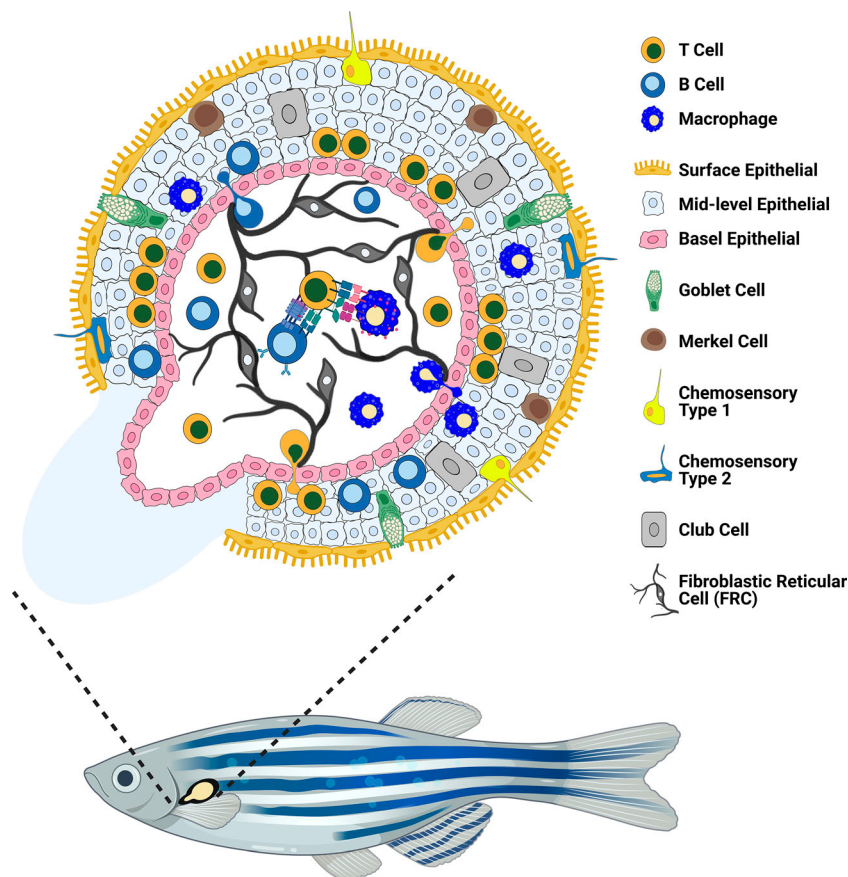
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throughout the zebrafish skin called the “tessellated lymphoid network” (Robertson et al., 2023). The tessellated lymphoid network facilitates T cell migration to infected sites and the detection of local antigens through a mechanism of antigen scanning and local activation. Finally, fish have an intricate system of MALTs that defend exterior and interior surfaces against pathogens. These MALTs comprise large numbers of T cells and are found in the gut, gills, skin, and orobranchial cavities (Kong et al., 2019; Resseguier et al., 2023; Salinas, 2015; Yu et al., 2019). Yet, unlike mammals, fish MALTs lack distinct lymphoid follicles and instead have immune cells scattered along the mucosal epithelia. Although MALTs contain small numbers of B cells and

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Schematic of the zebrafish ALO. The ALO is composed of an outer cortex divided into three distinct epithelial layers and an inner medulla characterized by a network of thin, elongated fibroblastic reticular cells (FRCs). The dense FRCs in the medulla facilitate the migration of T, B, and macrophage cells and increase the chances of communication and interaction between these cell types. The ALO's cortex also harbors goblet, Merkel, and chemosensory cells, suggesting that the ALO may also coordinate immune responses with environmental cues.

macrophages, the description of immune cell interactions and the education of B cells akin to what occurs at germinal centers has not been widely reported. To date, secondary lymphoid organs with shared similarity to mammals have yet to be fully described in teleost fishes, including the zebrafish.

Here, [Castranova et al. \(2025\)](#) report a previously unidentified and uncharacterized secondary lymphoid organ in the zebrafish, which they aptly call the axillary lymphoid organ (ALO). The ALO develops as a bilateral protrusion at the base of the zebrafish pectoral fin beginning at ~30 days after fertilization, coincident with the establishment of a fully functional adaptive immune system ([Miao et al., 2021](#)). Ultrastructure analyses revealed a highly complex structure of the ALO, comprising a stratified cortex with three layers of epithelia that are highly infiltrated by immune cells. The inner medulla is packed with blood and

lymphatic vessels, akin to the structure of mammalian lymph nodes ([Grant et al., 2020](#)). Single-cell RNA sequencing and subsequent live-cell imaging revealed a profound enrichment of lymphocytes in the ALO, with over 41% of the cellular composition being lymphocytes. Excitingly, T and B lymphocytes, along with macrophages, are juxtaposed in close proximity within the ALO and move dynamically throughout both the cortex and the medulla's dense fibroblastic reticular cell network, providing ample opportunities for cell:cell communication and contact between these cell types within the ALO. The detection of these latter movements in the ALO is similar to that found in the fibroblastic reticular cell networks of mammalian lymph nodes ([Grant et al., 2020](#)). Curiously, the ALO is also linked with lymph vessels and a circulatory network that may facilitate direct migration of thymic T cells into the ALO. In

total, the structural organization of the ALO suggests that it is an important new secondary lymphoid organ and may contain the long-sought-after “germinal center-like” sites in the zebrafish, thereby providing opportunities for antigen-presenting cells to communicate with T cells and subsequently activate B cell maturation and antibody production.

The exceptional accessibility and architectural characteristics of the ALO also provide new and unprecedented possibilities for real-time, dynamic visualization of immune processes that until now have been difficult to image in living vertebrates. Imaging will likely include activation and migration of lymphocytes; the trafficking of immune cells between the bloodstream, lymphatic vessels, and adjacent environments; and interactions that occur between innate and adaptive immune cells and pathogens. The ALO is also easily amenable to experimental manipulation, allowing one to directly apply pathogens or therapeutic agents onto the organ. Finally, the ALO also contains a wide array of sensory and secretory cells. These include Merkel cells that contain mechanoreceptors that detect light touch and pressure; chemosensory cells that detect chemicals, food, and predators in the water; and club cells that secrete “fright substance” (Schreckstoff) upon injury, which acts as a fear-eliciting compound to adjacent schooled fish. This distinctive cell composition, along with the proposed germinal center-like activity of the ALO, suggests that this secondary lymphoid organ may also coordinate environmental cues with immune response, akin to the neuro-immune cross talk mechanisms reported in mammals ([Godinho-Silva et al., 2019](#)).

The discovery of the ALO in zebrafish is exciting, but it also raises several questions for the future. (1) What is the specific role of this organ in the zebrafish immune system? (2) How is it functionally similar or different from other mammalian secondary lymphoid organs? (3) Can it serve as a model for human immune responses and disease? With the powerful approaches of live-cell imaging, chemical genetics, transgenesis, and gene inactivation tools available in the zebrafish, the field is well positioned to answer these questions and ultimately uncover new roles for the ALO in coordinating immune responses. These studies are expected to inform our understanding of immune cell coordination and function within secondary lymphoid organs that will likely

extend to a wider array of organisms, including mammals.

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References

- Björger, H., and E.O. Koppang. 2021. *Immunogenetics*. <https://doi.org/10.1007/s00251-020-01196-0>
- Castranova, D., et al. 2025. *J. Exp. Med.* <https://doi.org/10.1084/jem.20241435>
- Godinho-Silva, C., et al. 2019. *Annu. Rev. Immunol.* <https://doi.org/10.1146/annurev-immunol-042718-041812>
- Grant, S.M., et al. 2020. *J. Cell Sci.* <https://doi.org/10.1242/jcs.241828>
- Hu, C., et al. 2024. *Elife*. <https://doi.org/10.7554/eLife.92424>
- Kong, W.G., et al. 2019. *J. Immunol.* <https://doi.org/10.4049/jimmunol.1900863>
- Melo-Silva, C.R., and L.J. Sigal. 2024. *Cell. Mol. Immunol.* <https://doi.org/10.1038/s41423-024-01188-0>
- Miao, K.Z., et al. 2021. *Front. Cell Dev. Biol.* <https://doi.org/10.3389/fcell.2021.660969>
- Resseguier, J., et al. 2023. *Sci. Adv.* <https://doi.org/10.1126/sciadv.adj0101>
- Robertson, T.F., et al. 2023. *Proc. Natl. Acad. Sci. USA*. <https://doi.org/10.1073/pnas.2301137120>
- Salinas, I. 2015. *Biology (Basel)*. <https://doi.org/10.3390/biology4030525>
- Shibasaki, Y., et al. 2023. *Sci. Immunol.* <https://doi.org/10.1126/sciimmunol.adf1627>
- Yu, Y.Y., et al. 2019. *iScience*. <https://doi.org/10.1016/j.isci.2019.08.034>