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The constant exposure of the fish branchial cavity to aquatic pathogens must have driven local mucosal immune responses to be extremely important for their survival. In this study, we used a universal marker for T lymphocytes/natural killer cells (ZAP70) and advanced imaging techniques to investigate the lymphoid architecture of the zebrafish branchial cavity. We identified a new lymphoid organ, which we tentatively named "Nemausean Lymphoid Organ" (NEMO), situated below the pharynx, and closely associated with gill lymphoid tissues. Besides T/NK cells, NEMO is enriched in plasma/B cells and antigen-presenting cells embedded in a network of reticulated epithelial cells. Presence of activated T cells and lymphocyte proliferation but not V(D)J recombination or hematopoiesis, suggests a function as secondary lymphoid organ. In response to infection, NEMO displays structural changes including the formation of T/NK cells clusters. NEMO and gill lymphoid aggregates form a cohesive unit within a lymphoid network that extends throughout the pharyngo-respiratory area. Collectively, our findings reveal a new mucosal lymphoid organ reminiscent of mammalian tonsils that evolved in fish. Importantly, NEMO could clearly be identified in multiple teleost fish families.

## DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 104999
## TRANSCRIPTOMIC PROFILE OF THE GILLS WITH COMPLEX GILL DISEASE (CGD) LESIONS IN ATLANTIC SALMON (*SALMO SALAR*) FARMED IN A MARINE ENVIRONMENT

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The complex gill disease or disorder (CGD) is referred to a non-specific gill disease of farmed Atlantic salmon (Salmo salar) in the marine environment. In this study, we performed a transcriptomic profile of the gills with complex gill disease (CGD) lesions in Atlantic salmon sampled from seawater cage of a farm located in the fjords of the Aysén Region (Chile). For RNA-seq transcriptomic analysis, total RNA was obtained from the CGD portion of the gill and the Immediately Adjacent Tissue (IAT) using a pooling strategy (n= 3 pools per condition; n= 5 fish per pool). The differential gene expression (DEG) analysis was based on the negative binomial distribution using the DESeq2 package. We mapped our data to a total of 13,232 genes. We observed a different expression profile between CGD and IAT phenotypes. We found 332 down-regulated and 364 upregulated DEGs. In the functional enrichment analysis, we identified several processes associated with the immune response at innate (Toll like receptor cascade, complement cascade, Fcgamma receptor-dependent phagocytosis, antimicrobial peptides, among others), adaptative (MHC-I and MHC-II antigen presentation, co-stimulation by the CD28 family, signaling of B cell receptor, among others), and cytokine signaling level (Interferon signaling, signaling by interleukins, growth hormone receptor signaling, among others). These results indicate a complex network of interaction between the innate and adaptative immune system in response to CGD.

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## DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105000
## FUNCTIONAL CHARACTERIZATION OF HEXACORALLIAN PHAGOCYTIC CELLS, AND THEIR RESPONSE IN HEAT STRESS INDUCED BLEACHING

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Rising seawater temperatures have increased coral bleaching events worldwide, to the detriment of coral reefs. Previous research on coral heat stress-induced bleaching found that different immune genes were differentially regulated and suggested immune activation. Yet it is still uncertain if the immune response is a consequence of bleaching or of the heat stress itself. To test this, we first functionally characterize phagocytic cells in stony corals and sea anemones models. To test the role of the immune system in bleaching we have used two model systems: *Exaiptasia diaphana* – which can be reared with and without symbiotic algae, Symbiodiniaceae, and *Nematostella vectensis* - which lacks symbiotic algae. We examined the effect of increased temperature on phagocytic activity, as an indication of immune function, using flow cytometry. Our data shows that immune cell activity increases during heat stress, while small molecule pinocytosis remains unaffected. We observed an increase in cellular production of reactive oxygen species with increasing temperatures. We also found that the cellular immune activity was not affected by the presence of Symbiodiniaceae. This suggests that immune activity observed in heat-stress induced bleaching in corals is a fundamental and basic response independent of the bleaching effect. These results establish a foundation for improving our understanding of hexacorallian immune cell biology, and its potential role in coral bleaching.

## DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105001
## ADOPTIVE TRANSFER OF SENSITIZED LEUKOCYTES CONFERS PARTIAL PROTECTION AGAINST HERPESVIRAL HEMATOPOIETIC NECROSIS (HVHN) IN GINBUNA CRUCIAN CARP *CARASSIUS AURATUS LANGSDORFII* IMMUNIZED WITH A LIVE ATTENUATED VACCINE

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A live attenuated vaccine was developed against HVHN of goldfish, caused by cyprinid herpesvirus-2 (CyHV-2). However, the mechanism of immunity acquired after vaccination remains unknown. The objectives of this study are to identify susceptible hosts' immune responses and investigate the role of adoptive transferred sensitized leukocytes in protection against HVHN using the susceptible isogenic ginbuna. Goldfish and ginbuna (Wakin and S3n strain, respectively; BW 8 g) were vaccinated by the showering vaccination method (1:100 diluted vaccine; holding fish on the net for 10 s). Vaccinated

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fish were challenged with a virulent CyHV-2 by immersion after 21 days and the mortality was recorded. Meanwhile, the trunk-kidney and spleen leukocytes were isolated to determine CD8$\alpha^+$ lymphocyte dynamics. Antibody titer in serum was determined by ELISA using purified virus particles. In the adoptive transfer experiment, pooled whole trunk-kidney and spleen leukocytes of the vaccinated donor ginbuna (n=14) after 55 days were intraperitoneally injected ($3.5 \times 10^6$ cells/fish) into naïve recipient ginbuna (n=10). The serum of donor ginbuna was collected for ELISA. After one day, the donor and recipient ginbuna were challenged with a virulent CyHV-2. The vaccine was effective in both fish species against CyHV-2 challenge considering the increase of CD8$\alpha^+$ lymphocytes and ELISA antibody titer. The adoptive transfer experiment showed survival rate of donor, recipient and infected-control ginbuna at 80%, 40%, and 0%, respectively. Donor ginbuna had low seropositivity before CyHV-2 challenge. These results suggest that humoral and cell-mediated immunity play significant roles in the protective immunity induced by the vaccine.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105002**
**THE T CELL RECEPTOR REPERTOIRE OF THE AFRICAN LUNGFISH**

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T cell receptors (TCRs) are expressed on the T cell membrane in all jawed vertebrates. Two broad subsets of T cells are distinguished based on the TCR heterodimer expressed on their cell surface, αβ or γδ T cells. αβ T cells have diverse repertoires and recognize vast diversities of antigens. In contrast, γδ T cells exhibit reduced diversity in their TCR repertoire and are preferentially found in epithelial barrier tissues such as the skin where they play innate-like roles in tissue homeostasis and repair. African lungfish are the closest living taxon to all tetrapods and their skin undergoes drastic remodeling during estivation. In this study we annotated the TCR locus of the African lungfish *Protopterus annectens* using its newly published genome and found that its structure is similar to that of birds and mammals (TRV-TRDD-TRDJ_TRDC_TRAJ-TRAC) and different from that of teleosts. We characterized the spleen (central) and skin (peripheral) TCR repertoire in *P. dolloi* in two different free-swimming individuals and an estivated individual. We observed that that *P. dolloi* skin has a less diverse TCRγ and TCRδ repertoire characterized by a longer CDR3 region (9-19 amino acids) compared to skin TCRα and TCRβ, as well TCRγ and TCRδ in the spleen (8-17 amino acids). Estivated lungfish skin displayed an even more restricted TCR repertoire compared to controls. These results indicate that specialization of γδ T cells with innate-like functions in the skin predates the origin of tetrapods and that γδ T cells may be involved in skin immunity during estivation.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105003**
**T-CELLS IN THE CENTRAL NERVOUS SYSTEM OF HEALTHY AND NEUROTROPIC VIRUS (BETANODAVIRUS RGNNV) -INFECTED TELEOST *DICENTRARCHUS LABRAX***

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Evidences are increasing that vertebrate leukocytes play an active role in inflammation and immune defence of central nervous system (CNS), but the contribution of lymphocytes in cellular responses of the fish brain against pathogens is poorly known. Here we show first data on the presence and involvement of T cells and CD45$^+$-T cells during *in vivo* intramuscular infection of the Mediterranean sea bass *Dicentrarchus labrax* with the CNS-infecting virus betanodavirus (RGNNV, $5x10^5$ TCID/g). By employing the mAbs DLT15 for pan-T cells and DLT22 for CD45 (subtype R0)-T cells in IIF and IHC of leukocytes obtained from brain homogenates, we measured a mean content of 3,7±2.3% of DLT15-positive cells and 8.3±2.1% of DLT22-positive cells. After 6 days of RGNNV infection the percentages shifted to 7.2±3.1% of DLT15 and 11.6±4,1% of DLT22. An *in situ* analysis of brain sections by IHC showed in healthy fish the presence of immunoreactive pan-T cells and CD45-R0 T cells distributed in brain parenchima and enriched in anatomical sub-regions. After 6 days of RGNNV infection the number of both pan-T cells and CD45-R0 T cells revealed an evident increase, suggesting a recruitment and direct involvement of these leukocytes in brain antiviral response. The quantitative measurement of lymphocytes by computer-assisted IHC and analysis of T cell-related gene expression by 3' end RNA-sequencing from the CNS of control and virus-infected fish is in progress.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105004**
**PHAGOCYTIC CELLS IN THE INVERTEBRATE CHORDATE *BOTRYLLUS SCHLOSSERI* SHOW INFLAMMATORY AND REGENERATIVE PLASTICITY SIMILAR TO MAMMALIAN M1/M2 MACROPHAGES**

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Macrophages are vital phagocytic cells found in vertebrates and have essential functions in tissue homeostasis, immune responses, and tissue repair. They can be categorized into two phenotypes, M1 and M2, associated with inflammation/immune response and tissue regeneration/repair, respectively. Macrophages exhibit phenotypic plasticity, switching between these two states based on environmental cues, which plays a significant role in chronic inflammatory diseases and tumor progression. However, it is unclear if invertebrate phagocytes display similar plasticity and conserve molecular mechanisms and functional processes. To investigate this, we study phagocytic cells in the invertebrate chordate model organism, Botryllus schlosseri. We use fluorescent labeling and live cell imaging in the transparent Botryllus to observe and study phagocytic cell functions in real-time. The synchronized clearance of apoptotic cells, response to tissue injury, and vascular regeneration in Botryllus allow us to manipulate and characterize phagocytic diversity.
Through microscopy and mRNA sequencing, we discovered that the phagocytic cells in Botryllus exhibit similar characteristics and utilize comparable surface markers and communication pathways as mammalian macrophages. These cells detect and migrate to infected or injured areas, break down engulfed material through autophagy, and express markers associated with both M1/M2 types of macrophages, depending on the blastogenic stage. Live imaging revealed their roles in immune responses, maintaining homeostasis, wound healing, and eliminating harmful cells. This indicates that these cells respond to specific signals and contribute to various functions based on the situation. Our findings suggest that macrophages have evolved to encompass multiple roles within organisms, with distinct types emerging as complexity.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 105005**
**CHARACTERIZATION OF ANTIVIRAL IMMUNE RESPONSES IN CARP CELL LINES TO INFORM ON THE DEVELOPMENT OF FASTIDIOUS CARP VIRUS INFECTION MODESL**

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Global aquaculture production is one of the fastest growing food sectors with common carp (*Cyprinus carpio*) representing 5.2% of aquatic production. Health management is key to sustainable production. However, viral