DEPLETION OF IGT$^+$ B CELLS IN RAINBOW TROUT POINTS TO AN ESSENTIAL ROLE OF IGT IN MICROBIOME HOMEOSTASIS AND CLEARANCE OF MUCOSAL PATHOGENS

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ABSTRACT

The vast majority of fish pathogens enter their host through mucosal surfaces. The skin, gut and gill represent the largest mucosal surfaces in fish and they all contain a mucosal-associated lymphoid tissue (MALT). We have previously demonstrated that pathogen-specific mucosal antibody responses in the gut, skin and gill are overwhelmingly mediated by the IgT antibody class. In addition, we have shown that IgT is the main immunoglobulin isotype coating commensal bacteria and thus, it plays a key function in immune exclusion. While these IgT activities point to a pivotal role of this immunoglobulin in teleost mucosal immunity, whether IgT is required for pathogen clearance and microbiota homeostasis remains to be demonstrated. To address this critical question we have developed a unique IgT$^+$ B-cell depletion trout model. Upon one depletion treatment, IgT$^+$ B cells from all analyzed mucosal and systemic lymphoid organs were depleted by over 95% for a 3-4 week period. The application of an additional depletion treatment executed at day 21 was able to extend the depletion status by 3-4 weeks. Interestingly, the % of IgM$^+$ B cells did not change throughout the 7 week depletion period. Upon IgT$^+$ B-cell depletion, fish where sublethally challenged with *Ichthyophthirius multifiliis* or *Flavobacterium columnare*. After two weeks post-challenge, a significant percentage of mortality occurred in the IgT$^+$ B-cell depleted groups (25-50%). Critically, pathogen load was dramatically higher in the IgT$^+$ B-cell depleted groups when compared to control fish. Moreover, IgT coating of microbiota had for the most part disappeared 3 weeks post-depletion treatment. More importantly, the microbiome at mucosal surfaces was significantly changed. Interestingly we could never observe IgM or IgD compensatory responses against the pathogens or commensals. In conclusion we demonstrate that IgT is essential for pathogen clearance at mucosal surfaces, and it plays a critical role in the maintenance of microbiota homeostasis. This represents the first non-mammalian model in which a specialized mucosal immunoglobulin and the B cells producing it are depleted. Importantly, this novel IgT$^+$ B-cell depletion model will be critical to understand further the role of IgT in host-pathogen interactions at fish mucosal surfaces. At the more practical level, this new capacity to elucidate whether a pathogen requires mucosal IgT responses for its

clearance will direct efforts to the development of vaccines that not only stimulate systemic IgM but also mucosal IgT responses against that particular pathogen.

KEYWORDS
IgT, IgT$^+$ B cells, rainbow trout, microbiota, mucosal immunity

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