Topic Introduction

Experimental Platform Using the Amphibian *Xenopus laevis* for Research in Fundamental and Medical Immunology

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The amphibian *Xenopus* constitutes a powerful, versatile, and cost-effective nonmammalian model with which to investigate important contemporary issues of immunity relevant to human health such as ontogeny of immunity, self-tolerance, wound healing, autoimmunity, cancer immunity, immunotoxicology, and adaptation of host immune defenses to emerging pathogens. This model system presents several attractive features: an external developmental environment free of maternal influence that allows for easy experimental access from early life stages; an immune system that is remarkably similar to that of mammals; the availability of large-scale genetic and genomic resources; invaluable major histocompatibility complex (MHC)-defined inbred strains of frogs; and useful tools such as lymphoid tumor cell lines, monoclonal antibodies, and MHC tetramers. Modern reverse genetic loss-of-function and genome-editing technologies applied to immune function further empower this model. Finally, the evolutionary distance between *Xenopus* and mammals permits distinguishing species-specific adaptation from more conserved features of the immune system. In this introduction, the advantages and features of *Xenopus* for immunological research are outlined, as are existing tools, resources, and methods for using this model system.

INTRODUCTION

Xenopus has firmly been established as the model of choice for fundamental and medical immunological research. Its immune system is one of the most comprehensively studied besides those of mammals and chickens, and it shows remarkable similarity to that of mammals (for reviews, see Robert and Ohta 2009; Robert and Cohen 2011; Robert 2016). Indeed, the fully sequenced and annotated genomes of two *Xenopus* species, *X. tropicalis* and *X. laevis*, have provided compelling evidence that there is a high degree of similarity between the gene repertoires of *Xenopus* species and those of mammals, including humans (Hellsten et al. 2010; Session et al. 2016).

Over a century of studies have helped to characterize the *Xenopus* immune system. It contains primary lymphoid organs, as in mammals, including the thymus (the site of T-cell differentiation) and the spleen (a reservoir of antibody-producing B cells, T cells, and other leukocytes). As in mammals, *Xenopus* innate immune cells include neutrophils, basophils, eosinophils, monocytes, macrophages, and natural killer (NK) cells, and the adaptive T and B lymphocytes express a wide repertoire of somatically generated receptors that require recombination-activating genes (RAGs) 1 and 2. The development, activation, and restriction of T cells are directed by polymorphic molecules of the major histocompatibility complex (MHC). MHC class I molecules (for cytotoxic responses) and MHC class II molecules (for helper T cell responses) have been identified (for reviews, see Du Pasquier et al. 1989;

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Robert and Ohta 2009), as have nonpolymorphic MHC class I-like molecules important for innate-like T (iT) cells, which express a very limited repertoire of T cell receptors compared to conventional T cells (Robert and Edholm 2014). iT cells are also found in humans (e.g., iNKT cells and mucosal-associated T [MAIT] cells) and are of growing medical interest (for reviews, see Robert and Edholm 2014; Castro et al. 2015).

XENOPUS AS AN IMMUNOLOGICAL MODEL

Because of its mammalian-like immunity and the fact that it is easy to propagate and experimentally manipulate in the laboratory, *Xenopus* is ideally suited to serve as a powerful model system for multiple areas of immunological research, some of which are listed below.

- ***Model for developmental immunology:*** In contrast to humans and mice, anuran amphibians such as *Xenopus* embryos develop externally, thus free of maternal influences. This allows easy access for experimental manipulation and visualization at all stages of development and provides a unique model for perinatal immunology. Comparison of the immune system at two distinct life stages, larval and adult, affords intriguing insights into immune function specialization (as summarized in Fig. 1). In addition, drastic developmental remodeling occurs during metamorphosis in *Xenopus*, which also affects the immune system. The larval thymus undergoes histolysis, losing most of its thymocytes, and becomes repopulated at metamorphosis completion by new stem cells that differentiate into "adult-type" T cells (for review, see Rollins-Smith 1998). To prevent autoimmunity, T-cell education needs to establish tolerance against the many new adult-type proteins. T-cell-deficient animals can easily be generated by sublethal γ-irradiation or by thymectomy within the first week after fertilization, before the immigration of stem cells. In addition, an athymic strain has been generated by genome editing in *X. tropicalis* (Nakai et al. 2016). Therefore, *Xenopus* is a valuable experimental model to study T-cell ontogeny. With advances in genomic, genetic, and genome-editing technologies, *Xenopus* will offer new approaches to reveal the functions of immunologically relevant genes and immune cells.
- ***Model for iT cell immunity:*** To date *Xenopus* is the only species besides mammals where an immune surveillance system based on nonpolymorphic MHC class I-like molecules directing the development and function of iT cells has been characterized. Concomitant with a naturally

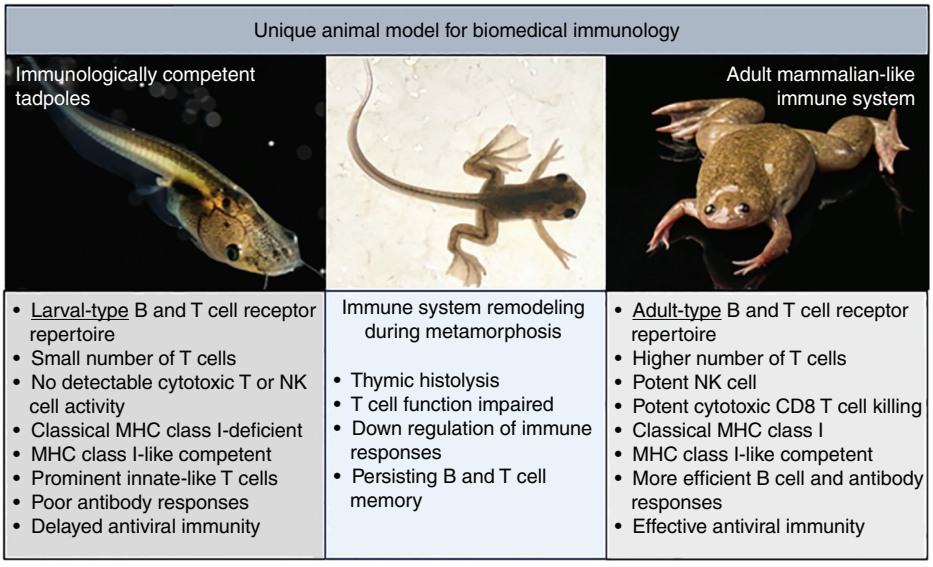

FIGURE 1. Schematic overview of the major developmental steps of the *Xenopus laevis* immune system.

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deficient classical MHC class I function and a diversification of MHC class I-like genes, tadpoles rely on an adaptive immune system dominated by iT cells. In contrast, mainly conventional T cells with a minor iT cell fraction are present in adult frogs. This provides a unique model system (and an alternative to mice) to investigate in vivo the role of these important T-cell types in antimicrobial and tumor immunity as well as in immune homeostasis and autoimmunity (for reviews, see Robert and Edholm 2014; Castro et al. 2015; Banach and Robert 2017a).

- ***Model for immune tolerance:*** *Xenopus* is an attractive comparative model to explore self-tolerance because allotolerance to minor H antigens on transplanted adult skin can be induced just before or during metamorphosis when immune regulation is temporarily altered (Flajnik et al. 1987). During this developmental period, it is possible to establish specific tolerance to minor H antigens that persists after metamorphosis in adult frogs and that relies on a nondeletional ("split") anergic-like process (for review, see Houssaint and Flajnik 1990). Genes encoding MHC molecules are also differentially regulated in tadpoles and adult frogs (Rollins-Smith 1998). The change in MHC gene expression during metamorphosis coincides with new T-cell differentiation in the thymus. Thus, the amenability of *Xenopus* to experimental manipulation (e.g., thymectomy, blocking, or accelerating metamorphosis) from early development is convenient for addressing questions about MHC restriction, autoimmunity, and the development of self-tolerance that are challenging to study in mammalian models.
- ***Model for tumor immunity and tumorigenesis:*** *X. laevis* and more recently *X. tropicalis* have proven instrumental for exploring novel and innovative approaches for cancer biology and immunotherapy (for reviews, see Banach and Robert 2017a; Hardwick and Philpott 2018). Thymic lymphoid tumor lines, which are tumorigenic after transplantation into compatible MHC-defined inbred strains, or isogenic clones can be used to investigate tumor immunity (Banach and Robert 2017a). Tadpole transparency, temperature tolerance, and accessibility are ideal for studying the tumor microenvironment by intravital microscopy in real time (Haynes-Gimore et al. 2015). The implementation of genome-editing technology in *Xenopus*, especially *X. tropicalis*, is another promising avenue to identify and characterize mutations in human oncogenes, those relevant for cancer development (for review, see Dimitrakopoulou et al. 2019). As such, the comparative tumor immunity model in *Xenopus* can significantly contribute to designing and testing novel immunotherapeutic approaches against cancer.
- ***Model for immunity to important infectious diseases:*** *Xenopus* plays a key role in understanding infectious diseases that plague amphibians worldwide and contribute to their decline. Notably, *X. laevis* is the leading experimental model to study host defense and pathogenesis during ranavirus infection and to evaluate the contribution of immunocompromised animals in disease dissemination (for reviews, see Chen and Robert 2011; Chinchar and Waltzek 2014). Comparative studies (e.g., of tadpole vs. adult resistance to ranavirus or mycobacterial infections [for review, see Hyoe and Robert 2019] or of *X. tropicalis* vs. *X. laevis* resistance to chytrid fungal infections) can provide novel fundamental insights into virulence and immune escape mechanisms (for review, see Rollins-Smith et al. 2009; Grogan et al. 2018). The *Xenopus* skin secretes multiple antimicrobial peptides that are highly active against human immunodeficiency virus (HIV) as well as against many human gram negative and positive bacteria, which is of high biomedical relevance (for reviews, see Zasloff 1987; Rollins-Smith et al. 2009).
- ***Model for wound healing, inflammation, and regeneration:*** The *Xenopus* tadpole life stage offers an advantageous system for regeneration research (for reviews, see Slack et al. 2008; Chen et al. 2014; Li et al. 2016). The amputation of the tadpole's limb or tail results in the regeneration of a completely new tail that is fully functional. Immune cells (e.g., macrophages) and an inflammatory immune response are critically involved in this process as well as during wound healing. The transparency of tadpoles is ideal for intravital studies (Haynes-Gimore et al. 2015; Paredes et al. 2015), and they are amenable to single-cell transcriptomics (Aztekin et al. 2019).
- ***Model for immunotoxicology:*** Because of its external development free of maternal influence, *Xenopus* is a sensitive and cost-effective model system to investigate both acute and chronic

effects resulting from early life exposure to water pollutants (e.g., endocrine disruptors of immune homeostasis and of immune responses to infectious diseases), which is gaining interest and attention from the public and the scientific community (Robert et al. 2018; Nagel et al. 2020).

TOOLS AND RESOURCES FOR *XENOPUS* IMMUNOLOGY RESEARCH

Importantly, tools (antibodies and cell lines) as well as genetically defined inbred clones and transgenic strains that are indispensable for immunological research are available in *Xenopus* and can be obtained at the *Xenopus laevis* Research Resource for Immunobiology (https://www.urmc.rochester.edu/microbiology-immunology/xenopus-laevis.aspx). These include monoclonal antibodies (mAbs) specific for *X. laevis* NK, B, and T cells and immune-related molecules such as CD8 and MHC (Robert and Ohta 2009). It is noteworthy that some of these mAbs, including the anti-IgY (11D5) and anti-CD8 (AM22), also cross-react with *X. tropicalis*. Furthermore, given the support of the genome and inbred strains developed in *X. tropicalis* (and available in the Japanese and U.S. stock centers), information obtained in *X. laevis* about gene expression and gene loss-of-function can be easily transposed.

The accompanying protocols include convenient immune assays adapted to *Xenopus* such as flow cytometry analysis (Protocol: **Flow Cytometric Analysis of *Xenopus* Immune Cells** [Edholm 2018]) and antibody response analysis by enzyme-linked immunosorbent assay (ELISA) (Protocol: **Assessing Antibody Responses to Pathogens or Model Antigens in *Xenopus* by Enzyme-Linked Immunosorbent Assay (ELISA)** [De Jesús Andino and Robert 2019]). These are followed by the use of invaluable MHC-defined inbred strains and cloned animals for in vivo immune assays such as skin grafting (Protocol: **Skin Grafting in *Xenopus laevis*: A Technique for Assessing Development and Immunological Disparity** [Izutsu 2019]), tumor transplantation (Protocol: **Collagen-Embedded Tumor Transplantations in *Xenopus laevis* Tadpoles** [Banach and Robert 2017b]), elicitation of peritoneal leukocytes (Protocol: **Elicitation of *Xenopus laevis* Tadpole and Adult Frog Peritoneal Leukocytes** [Grayfer 2018]), and adoptive cell transfer (Protocol: **Adoptive Transfer of Fluorescently Labeled Immune Cells in *Xenopus*** [Rhoo and Robert 2019]). Notably, adoptive cell transfer and tumor transplantation can also use MHC defined isogenic clones (e.g., LG-6 and LG-15). These clones are produced by gynogenesis from diploid eggs of *X. laevis* x *X. gilli* interspecies hybrids (Kobel and Du Pasquier 1975; Du Pasquier et al. 1977). Since irradiated sperm is used, only genetically identical females are produced. Finally, assays not easily achieved in mammalian models are described, including the convenient generation of immune-deficient animals by sublethal irradiation (Protocol: **Lymphocyte Deficiency Induced by Sublethal Irradiation in *Xenopus*** [Rollins-Smith and Robert 2019]), by thymectomy at an early developmental stage (Protocol: **Larval Thymectomy of *Xenopus laevis*** [Mashoof et al. 2018]), and by RNAi-mediated loss of function by transgenesis using the I-SceI meganuclease (Protocol: **RNAi-Mediated Loss of Function of *Xenopus* Immune Genes by Transgenesis** [Edholm and Robert 2018]).

CONCLUSION

In summary, the combination of attractive and unconventional features with conserved mammalian-like immunity empowers *Xenopus* as an influential multifaceted experimental platform for exploring fundamental (e.g., evolution and ontogeny) as well as medical (e.g., cancer, inflammation, and infectious disease) aspects of immunity.

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