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Review

The genus *Xenopus* as a multispecies model for evolutionary and comparative immunobiology of the 21st century

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ABSTRACT

The *Xenopus* model for immunological research offers a collection of invaluable research tools including MHC-defined clones, inbred strains, cell lines, and monoclonal antibodies. Further, the annotated full genome sequence of *Xenopus tropicalis* and its remarkable conservation of gene organization with mammals, as well as ongoing genome mapping and mutagenesis studies in *X. tropicalis*, add a new dimension to the study of immunity. In this paper, we review uses of this amphibian model to study: the development of the immune system; vascular and lymphatic regeneration; immune tolerance; tumor immunity; immune responses to important emerging infectious diseases; and the evolution of classical and non-classical MHC class I genes. We also discuss the rich potential of the species with different degrees of polypoidy resulting from whole genome-wide duplication of the *Xenopodinae* subfamily as a model to study regulation at the genome level.

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1. Introduction

Greg Warr has long been an ardent supporter of comparative immunology and science education. This statement is easily validated by his research program, his training of graduate and postdoctoral students, his leadership role as the editor of DCI and by his current position at the NSF. Early in 2002, Greg organized a remarkable workshop[1] in Charleston, SC entitled "Evolutionary Immunobiology: New Approaches, New Paradigms" that led to a white paper for the NSF (Warr et al., 2003). The objective of this workshop was to: provide an overview of the current knowledge in the field of comparative immunology, reveal areas and problems of future potential high impact, and identify the needs for the continued development of this discipline. As recognized in the white

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paper (Warr et al., 2003) and in a meeting report prepared by Du Pasquier and Courtney Smith (2003), the richness and complexity of defense systems of plants and animals far exceeds earlier predictions. This realization has been amply justified by research during the past few years. Indeed, the diversity of solutions utilized by organisms during evolution to control pathogens and tumors seems boundless. The dilemma recognized during the workshop and one that still remains unresolved is how to best deal with such a diversity and complexity.

A question that most comparative immunologists face soon or later is what is the relevance of studying the immune system of a "funny creature" (in the parlance of Bill Clem who co-edited DCI with Greg) rather than focusing all efforts and resources to studying the immune system of *Homo sapiens* and its immune alter ego, *Mus musculus*. We, as well as Greg, strongly believe that it is in fact of considerable value to explore a variety of nonmammalian species from the whole tree of life not only as models for better understanding the human condition but also because of the intrinsic value of knowing how the splendid diversity of organisms have adapted to their environment and have coped successfully with parasites, microbial pathogens, and malignant cell transformations.

The development of technologies such as genomics and proteomics during the last decade has opened new frontiers. The numerous genome projects associated with these new technologies such as deep-sequencing and bioinformatics provide boundless novel opportunities to explore biological diversity and from our particular perspective, the diversity of immunity. Therefore, since this special edition of DCI represents a tribute to Greg's sustained effort to promote our field, advocate the use of new technologies and to some extent, chart the direction of our journal, we think it is appropriate to illustrate the potential of a new age of comparative immunology with our animal model of choice, species of the *Pipidae*. This amphibian family includes *Xenopus laevis* and its sister species, *Xenopus tropicalis*, whose genome has now been fully sequenced and annotated (Robert and Ohta, 2009; Hellsten et al., 2010). The *Pipidae* is composed of species with various degrees of polyploidy (2–12*N*) (Evans, 2008). Thus, the *Pipidae* family, and especially the *Xenopodinae* subfamily, is a unique group among vertebrates owing to their postulated evolutionary emergence by genome duplication. For a more comprehensive account of the taxonomy, ecology, behavior, genetics, immunology, sensory physiology, and evolution of these amphibian taxa the reader can consult the monograph of reference edited by Tinsley and Kobel (1996). We think that the combination of the long term extensive characterization of the immune system of *X. laevis*, the ongoing genetic and genomic characterization of *X. tropicalis*, and the availability of a set of species with various degrees of polyploidy, in contrast to other models based on a single species, brings the possibilities for investigation to a new level. In this review, we will first present a short overview of the actual potential of the *Xenopus* model. Some of this information has also been discussed in the 2009 *Xenopus* Community White Paper 2009 prepared for the National Institutes of Health (http://xlaevis.cpsc.ucalgary.ca/community/xenopuswhitepaper.do). We will then discuss the future promises of the extended *Xenopodinae* model using as an example our recent work on nonclassical MHC class I genes.

2. Existing potential of the *Xenopus* model for comparative immunology

X. laevis continues to provide a powerful nonmammalian comparative model with which to study many facets of immunity. These include: humoral and cell-mediated immunity in the context of MHC restricted and unrestricted recognition; ontogeny; phylogeny; and defense against tumors, viruses, fungi and bacteria (reviewed in Robert and Ohta, 2009; Du Pasquier et al., 1989). Notably, the *X. laevis* model offers a collection of invaluable research tools including MHC-defined clones, inbred strains, cell lines (including lymphoid tumor, fibroblast and kidney cell lines), and mouse monoclonal antibodies specific for a variety of *Xenopus* cell surface makers (e.g., general leukocytes, pan T cells, CD8, NK, IgM, IgY, IgX, IgL, MHC class I, and class II). All these reagents, tools and animals, as well as related information, are available through a *X. laevis* research resource for immunobiology (http://www.urmc.rochester.edu/mbi/resources/Xenopus/). Additional *Xenopus* resource can be found on Xenbase (http://xlaevis.cpsc.ucalgary.ca/common/).

Finally, the annotated full genome sequence of *X. tropicalis* and its remarkable conservation of gene organization with mammals, as well as ongoing genome mapping and mutagenesis studies in *X. tropicalis*, add a new dimension to the study of immunity. In this paper, we will succinctly review some salient uses of this *Xenopodinae* model.

2.1. Model to study the development of the immune system

One of the earliest (and still important) scientific uses of *X. laevis* has been as a tool to understand embryogenesis and subsequent stages of development (reviewed in Heasman, 2006). From our comparative perspective, *X. laevis* has taught us much about the early ontogeny of the immune system. *X. laevis* has all the lineages of hematopoietic cells that mammals have. Unlike mammals, however, early developmental stages of *X. laevis* are free from maternal influence, and are easily accessible and amenable to experimentation. This provides an ideal system to study early commitments and fates of myeloid and lymphoid lineages (Suzuki et al., 2004; Marr et al., 2007). For example, a primitive myeloid cell population arising in the anterior ventral blood island at the end of the neurula stage has been recently characterized in *Xenopus* embryos. During the next 6–8 h of development (i.e., early tail bud stages), these cells migrate and populate the entire embryo (Costa et al., 2008). These migratory cells are the earliest differentiated blood cells described to date and their formation occurs well before both the differentiation of primitive erythrocytes (previously thought to be the earliest blood cells to differentiate), and the formation of a vascular network. Moreover, these primitive myeloid cells, which are the only cells with potential immunological function in the early embryo, are quickly and efficiently recruited to wounds over large distances before the establishment of functional vasculature (Chen et al., 2009). The *Xenopus* system has the additional advantage of the accessibility of the thymus early in development. Indeed, thymectomy can be efficiently performed in *Xenopus* at early developmental stages (before the migration of stem cells) to generate T cell-deficient animals (reviewed in Horton et al., 1998). Similar to the use of nude or RAG knockout mice (Mak et al., 2001), T cell-deficient *Xenopus* are critical for studying the role of T cells in transplantation and tumor immunity. Combined with MHC-defined stains and clones, further *in vivo* characterization of T cell effector subsets (e.g., cytotoxic CD8 T cells) by adoptive transfer is possible.

Although other animal models (e.g., zebrafish) also make it possible to access immune tissues early in development free of maternal influence, *Xenopus* with its second developmental period during metamorphosis provides a truly unique experimental model to study immune differentiation, regulation, and self-tolerance. During metamorphosis, the larval thymus loses most of its lymphocytes and a new differentiation occurs from a second wave of stem cell immigration; this results in completely distinct adult immune system (Du Pasquier and Weiss, 1973; Bechtold et al., 1992; Turpen and Smith, 1989). Notably, autoimmunity against the many new adult-specific proteins needs to be prevented by a new balance of self-tolerance through T cell education (Flajnik et al., 1987).