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Cells of cutaneous immunity in *Xenopus*: Studies during larval development and limb regeneration

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Abstract

The anuran *Xenopus laevis* is an experimental model for vertebrate development, immunology, and regenerative biology. Using histochemistry and immunohistochemistry (IHC) we examined embryonic, larval, and postmetamorphic *Xenopus* skin for the presence of dendritic cells (DCs), Langerhans cells (LCs), and dendritic epidermal T cells (DETCs), all components of cutaneous immunity that have been implicated in skin repair and regeneration. Cells expressing three markers for dendritic and Langerhans cells (formalin-resistant ATPase activity, major histocompatibility complex [MHC] class II antigens, and vimentin) and having morphology like that of these cells first appeared during late embryonic stages, becoming abundant by prometamorphosis. Cells positive for these markers were also numerous in the wound epithelia of regenerating hindlimbs at both early and late larval stages. Cells tentatively identified as DETCs were found, beginning at early larval stages, using IHC with antibodies against heterologous CD3ε chain and T-cell receptor δ. Further characterization and work with the putative DCs, LCs, and DETCs demonstrated here will allow not only greater understanding of the amphibian immune system, but also further elucidation of regenerative growth and scarring.

Keywords: *Xenopus*; Cutaneous immunity; Dendritic cells; Langerhans cells; DETCs; Limb regeneration; Wound healing; Scarring

1. Introduction

Recent work has produced a greatly increased appreciation for the importance and variety of immune components in the skin, the usual first line of defense against both environmental pathogens and physical injuries. Cutaneous immunity not only includes innate and adaptive mechanisms for defense of the organism [1,2] but also determines the quality of wound repair on a spectrum from excessive scarring to perfect skin regeneration [3,4]. For amphibians the protective and reparative roles of the cutaneous immune system are of particular interest given the role of skin infections in the decline of amphibian populations worldwide [5–7] and the ability of many urodeles and larval anurans to heal full-thickness skin wounds with little or no

Abbreviations: ATPase, adenosine triphosphatase; DC, dendritic cell; DETC, dendritic epidermal T cell; IHC, immunohistochemistry; LC, Langerhans cell; MHC, major histocompatibility complex; TCR, T-cell receptor

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scarring and to fully regenerate lost appendages [8–10].

The quality of skin wound healing in mammals is affected by immigrant cells such as neutrophils and monocytes and also by resident cells, including dendritic epidermal T cells (DETCs or $\gamma\delta$ T cells [11,12]) and two cells derived originally from circulating monocytes: dendritic cells (DCs) of dermis and Langerhans cells (LCs) in epidermis [3,13,14]. In light of the growing body of evidence that these cells affect tissue repair while modulating the peripheral immune response, what roles might they play in the remarkable capacity of amphibians for scarless wound healing and regeneration? Answering such questions is hampered by the paucity of basic information about amphibian DCs, LCs, and DETCs and their formation during development. Du Pasquier and Flajnik [15] reported that cells expressing major histocompatibility complex (MHC) class II antigens and resembling DCs and LCs morphologically appear in skin of the anuran *Xenopus laevis* by midlarval stages and become much more numerous at metamorphosis. Similar cells with dendritic appearance were demonstrated by ATPase histochemistry in adult *Rana* skin [16]. A subpopulation of ATPase-positive cells in both dermis and epidermis was subsequently found to co-express class II antigens [17], suggesting selective maturation and activation of these cells in a manner similar to that described for mammals [1]. Intraepithelial lymphocytes have been reported for the urodele digestive tract [18], but nothing is known about DETCs in amphibian skin.

Recently, expression of immunomodulatory factors and markers for immune cells has been found in *Xenopus* regeneration blastemas by subtractive hybridization [19,20] and microarray analysis [21,22]. In mammals, the tolerogenic function of "immature" DCs and LCs has been clearly established [23,24] and proteomic profiles have identified new markers for these cells, such as the cytoskeletal protein vimentin [25]. These findings, together with work linking immune regulation to organ regeneration in various mammalian systems [26,27], have prompted us to look again at cells of cutaneous immunity in *Xenopus* in an effort to better understand the amphibian capacity for regeneration and scarless healing. Using ATPase histochemistry and immunohistochemistry (IHC) with antibodies to various markers, including vimentin and CD3, we have demonstrated putative dermal DCs, LC-like cells, and putative DETCs and have examined the appearance of these cells in skin during larval development and hindlimb regeneration. The results indicate that such cells are available locally to modulate the outcome of the regenerative response.

2. Methods and materials

2.1. Animals

Larvae and juveniles of outbred *X. laevis* were reared from eggs fertilized in the lab or were purchased from Nasco (Fort Atkinson, Wisconsin, USA). Larvae were maintained in artificial pond water at 22 °C at a density of 10 per liter. Developmental stages are described according to the system of Nieuwkoop and Faber [28]. Anesthesia and all surgical procedures were performed in accordance with US government standards under the supervision of the institutional animal use committee.

2.2. ATPase histochemistry

Following euthanasia in buffered 0.03% benzocaine, sheets of intact skin were removed from larvae and newly metamorphosed animals, rinsed in phosphate-buffered amphibian saline, placed epidermis-up on glass slides, and dried at 37 °C for 30 min. Embryos were processed intact. Formaldehyde fixation and histochemical processing for adenosine triphosphatase (ATPase) activity were done as described by Castell-Rodriguez [17]. Controls were prepared in the same way but without ATP in the incubation medium.

2.3. Immunohistochemistry (IHC) of tissue whole-mounts

Larvae were fixed overnight in 80% methanol/20% DMSO at 4 °C and stored in 100% methanol at −20 °C if not used immediately. The tails were removed and the larvae were bisected midsagittally to provide experimental and control (no primary antibody) tissues from each. Tissues were rehydrated and washed in a 1:1000 solution of Tween 20 in phosphate-buffered saline (PBS). Subsequent indirect IHC procedures depended on the antigen studied, but always included 5-h rinses (with several changes) in PBS-Tween 20 and blocking with either serum-free protein block (Dako, #X0909) or 20% goat serum (Sigma) for 4 h before each antibody treatment or the color reaction. For *MHC II*