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Identification of Langerhans-like cells in the immunocompetent tissues of channel catfish, *Ictalurus punctatus*

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ABSTRACT

Dendritic cells (DCs) are the most powerful antigen presenting cells (APCs) that have a critical role in bridging innate and adaptive immune responses in vertebrates. Dendritic cells have been characterized morphologically and functionally in the teleost fish models such as rainbow trout, salmonids, medaka, and zebrafish. The presence of DCs with remarkable similarities to human Langerhans cells (LCs) has been described in the spleen and anterior kidney of salmonids and rainbow trout. However, there is no evidence of the presence of DCs and their role in channel catfish immunity. In this study, we assessed DC-like cells in the immunocompetent tissues of channel catfish by immunohistochemistry (IHC), flow cytometry and transmission electron microscopy (TEM). We identified Langerin/CD207$^+$ (L/CD207$^+$) cells in the channel catfish anterior kidney, spleen and gill by IHC. Moreover, we described the cells that resembled mammal LC DCs containing Birbeck-like (BL) granules in channel catfish spleen, anterior and posterior kidneys and gill by TEM. Our data suggest that cells with DC-like morphology in the immune related organs of catfish may share morphological and functional properties with previously reported DCs in teleost fish and mammals. More detailed knowledge of the phenotype and the function of catfish DCs will not only help gain insight into the evolution of the vertebrate adaptive immune system but will also provide valuable information for development and optimization of immunotherapies and vaccination protocols for aquaculture use.

1. Introduction

Dendritic cells (DCs) are the most powerful professional antigen presenting cells (APCs) that bridge, initiate, regulate, and control all innate and adaptive immune responses in vertebrates [1]. Although numerous studies are still mainly focused on mammalian DCs, reports on the morphological and functional characterization of DCs in teleost fish, the earliest vertebrates to develop functional adaptive immune responses, are scarce. Recently, DC-like cells, with T cell stimulatory capacities that revealed the properties of mature mammalian DCs have been identified in zebrafish (*Danio rerio*) [2,3]. Furthermore, functional cells with dendritic morphology, motility, phagocytic ability and strong T cell stimulatory properties have been identified in several other teleost fish species such as rainbow trout (*Oncorhynchus mykiss*) and medaka (*Oryzias latipes*) [4,5]. Co-stimulation is an important function of professional APCs to prime naïve T cells. The major co-stimulatory molecules such as CD80/CD86 and CD83 have been reported in zebrafish [6]. Shao et al. reported the functional conservation of surface phenotypic molecules on DCs in teleost fish as in mammals by demonstrating functional abilities of CD80/86/CD83/CD209$^+$ cells in teleost fish to promote CD4$^+$ naïve T cell activation [7].

Langerhans cells (LCs) are a distinct population of immature DCs in the epidermis of mammals, and this unique location at a barrier surface provides them with early access to skin pathogens, commensal organisms, foreign chemicals and epidermal self-antigens [8]. Langerhans cells play a sentinel role through their specialized function in antigen uptake and capture, their capacity to migrate to the secondary lymphoid organs and present antigens to

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specific T cells thus initiating acquired immune responses [9]. Langerhans cells are uniquely characterized by Birbeck granules (BGs), the organelles consisting of superimposed and zippered membranes. It was previously shown that Langerin is constitutively associated with BGs and is a potent inducer of membrane superimposition [10]. Induction of BGs is a consequence of the antigen capture function of Langerin, allowing routing into these organelles and providing access to a non-classical antigen processing pathway [11]. But how BGs influence the processing and presentation of antigens by MHC class I and class II is still not fully understood. A recent report showed that LCs mediated and enhanced cross-presentation when antigen was delivered through Langerin [12].

Several morphological studies identified the cell type strikingly resembling mammalian LCs within inflammatory gill lesions and the spleen of healthy fish based on the presence of BGs by using a commercial polyclonal antibodies (pAbs) developed against human Langerin [13–15]. In particular, Langerin/CD207$^+$ (L/CD207$^+$) cells were found in the spleen and anterior kidney of Atlantic salmon (*Salmo salar*) and rainbow trout [14,15]. Interestingly, BG-like granules were observed in the thymus and anterior kidney followed by the appearance within cells of the newly developed spleens in rainbow trout and Atlantic salmon [15]. Langerin-positive cells in healthy rainbow trout were seen predominantly in the spleen however, during microsporidial gill disease, the number of L/207$^+$ cells has been significantly increased in the spleen and anterior kidney [14].

Channel catfish (*Ictalurus punctatus*) is the most important aquaculture species in the United States, accounting for $352 million in 2014 [16]. Vaccination against catfish pathogens requires generation of T and B cell-mediated immunity controlled by professional APCs to eliminate infection and induce protective memory immune responses. Comprehensive understanding and knowledge of putative DC populations in catfish is not only important for health assessment and vaccine development, but also to study the evolution of the immune system. However, there is no evidence of the existence of DCs and their role in orchestrating innate and adaptive immunity in channel catfish. Therefore, the purpose of our research was to conduct a morphological assessment of DCs in immunocompetent organs of channel catfish. Here, for the first time, we report the presence of DC-like cells with the striking similarities to human LCs in the immune related organs of catfish. The identification of DC-like cells in catfish suggests that specialized APCs might share properties of the mammalian DCs to initiate and orchestrate innate and specific immune responses.

2. Materials and methods

2.1. Animals

Specific pathogen free (SPF) channel catfish fingerlings (five-six month old) and fry (one month old) were acquired from the fish hatchery at the College of Veterinary Medicine, Mississippi State University and maintained into flow-through tanks at 25–28 °C. Fish were fed with a floating catfish feed to satiety and acclimated for one week. In this present study, all fish experiments were carried out by a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Mississippi State University. The fish were euthanized in water containing 100 mg/L tricaine methanesulfonate (MS-222, Western, Chemical, Inc.). Samples were obtained as described below.

2.2. Cell preparation

Peripheral blood, anterior kidney, and spleen cell separation was performed as described previously [17]. Briefly, peripheral blood was collected from the caudal vein followed by centrifugation at 500 *g* and resuspended in Phosphate-buffered saline (PBS). Anterior kidney and spleen were dissected from the fish and placed in a sterile culture dish containing PBS. To obtain a single-cell suspension, tissue were minced with sterile forceps, repeatedly aspirated using a 1 ml syringe and passed through cell dissociation sieves (Sigma, St. Louis, MO). The resulting cell suspensions were washed and resuspended in PBS. Cell suspensions and peripheral blood were layered over Histopaque 1077 (Sigma) and centrifuged at 500 *g* for 30 min. Cells then were collected from the interface, washed three times in PBS, counted and assessed for viability by trypan blue exclusion.

2.3. Cell staining and flow cytometry

Monoclonal antibody specific to human L/CD207 conjugated with fluorochrome (R-PE) (PE Mouse Anti-human L/CD207, clone no: 2G3, BD Biosciences) and isotype-matched controls (Ig Lambda, clone no: 1-155-2, eBioscience) were used to identify the intracellular staining of L/CD207 in the immune related organs of catfish. Cells from peripheral blood, spleen and anterior kidney were fixed and permeabilized with BD Perm/Wash buffer (BD Biosciences) on ice for 20 min, and washed twice with BD Perm/WashTM buffer (BD Biosciences). Following fixation and permeabilization, cells were re-suspended with Streptavidin/Biotin block (Vector Laboratories) and incubated for 15 min in the dark and washed with BD Perm/WashTM buffer. After that, cells were incubated with R-Phycoerythrin-conjugated mAbs to human L/CD207 on ice for 30 min in the dark, and washed with BD Perm/WashTM buffer.

Catfish mononuclear cells were gated based on their relative size and granularity using forward and side scatters (FSC and SSC, respectively) with a FACSCalibur Flow Cytometer (Becton Dickinson). Immunofluorescent staining was analyzed using FlowJo 7.6.4 Software (Tree Star Inc.). The intensity of L/CD207 staining in catfish mononuclear cells was analyzed by using single histogram statistics.

2.4. Tissue preparation for immunohistochemistry

Spleen, anterior kidney, gill, and skin were isolated from the euthanized channel catfish and immediately fixed in 10% neutral buffered formalin for 24 h. Tissues were embedded in paraffin wax after the dehydration with a graded series of ethanol, and 5 or 6 sections were cut for per slide as described [14].

2.5. Immunohistochemistry (IHC)

Immunohistochemical staining of fingerling catfish spleen, anterior kidney, skin and gill paraffin slides for the presence of L/CD207$^+$ cells was performed with two different antibodies: purified human CD207-specific pAbs (R&D Systems, Inc.) and R-Phycoerythrin-conjugated mAbs specific to human L/207. Immunohistochemical staining with purified pAbs was conducted as described elsewhere with minor modifications [15]. In this study, antigen retrieval was examined by incubation of sections in target retrieval solution (DAKO) for 40 min at 100 °C. Following this incubation, sections were incubated in protein block (DAKO) for 1 h. After that, primary antibody was diluted at a concentration of 0.2 mg/ml (1:500 from stock solution), and sections were incubated in primary antibody for overnight at room temperature in a humid chamber. We used rainbow trout spleen as a positive control for L/CD207-specific staining. Also, negative control that was normal goat IgG (Vector Laboratories) was applied at the same concentrations as the primary antibody. After overnight incubation,