Fish & Shellfish Immunology 27 (2009) 365–368

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Langerin/CD207 positive dendritic-like cells in the haemopoietic tissues of salmonids

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ARTICLE INFO

Article history:
Received 6 November 2008
Received in revised form
19 December 2008
Accepted 11 January 2009
Available online 21 January 2009

Keywords:
Langerhans cells
Fish
Trout
Salmon
Spleen
Immunohistochemistry

ABSTRACT

The presence of dendritic cells in fish is studied with immunohistochemistry using a commercially available antibody developed against Langerin/CD207 present in human Langerhans cells. Langerin/CD207, a protein known to be associated with the development of Birbeck granules in human and murine systems, was found to be expressed within the cytoplasm of spleen and head kidney cells of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Reactivity was also observed within a few number of cells within the head kidney of Atlantic salmon, but not observed in any other tissues examined. Immunohistochemical results showed Langerin/CD207 reactivity in the cytoplasm of cells in Atlantic salmon and rainbow trout comparable to reactivity seen in human Langerhans cells. The results in this study further corroborate the presence of dendritic cells with remarkable similarities to human Langerhans cells in the spleens and to a lesser extent in head kidney of salmonids.

Fish are the earliest vertebrates to develop an adaptive immune system; it is believed that a sudden change "big bang" in immunity occurred in the jawed vertebrates, which equipped these animals with a combinatorial system required for adaptive immunity to occur [1]. The sudden requirement for these complex adaptive mechanisms to occur in jawed vertebrates is thought to be a response to predatory life [2]. Many studies have been done to understand mechanisms of adaptive immunity in fish and how they compare to mammals, however there is a lack of morphological studies on fish immune cells due to a lack of good cell markers [3]. Dendritic cells have emerged as important cell types for the induction of adaptive immunity in mammals; several subsets of these cells have been described in various organ systems serving to patrol for antigens [4]. Dendritic cells as of yet have not been identified in fish, although morphological studies have demonstrated the presence of a cell type in salmonids which strongly resembles Langerhans cells based on the presence of Birbeck-like granules. These dendritic-like cells occurred within gill inflammatory lesions caused by Microsporidial Gill Disease and as resident populations within the spleen of apparently healthy fish [5,6].

Langerin is a specific protein discovered in human Langerhans cells, which functions as an endocytic receptor and is a strong inducer for the development of Birbeck granules [7]. It has been demonstrated that Langerin localizes to Birbeck granules and that transfection of Langerin cDNA into fibroblasts leads to the development of Birbeck granules [7]. Langerin has also been shown to accumulate where Birbeck granules are formed [8] and without the presence of Langerin, Birbeck granules fail to develop within the cells [9]. Langerin has been demonstrated to be a highly specific marker of Langerhans cells and useful in diagnosing Langerhans cell histiocytoses in humans [10].

In this study spleen tissue from salmonids was examined by immunohistochemistry for the presence of Langerin/CD207. The fish used in this study included 15 g (range 10–20 g) pre-smolt Atlantic salmon, *Salmo salar* (Cardigan Fish Hatchery, Cardigan, PEI, Canada) and 50 g (range 40–60 g) rainbow trout, *Oncorhynchus mykiss* (Ocean Trout Farms, PEI, Canada). Fish were euthanized with an overdose of benzocaine and organs were immediately fixed in 10% neutral buffered formalin for 24 h, dehydrated through a graded series of ethanols and embedded in paraffin wax. Spleens were sampled from all fish and from 4 Atlantic salmon appropriate sections were taken to include thymus, gills, head kidney, trunk kidney, heart, liver, skin, and GI tract. Five μm sections were cut so that 3 sections of spleen would fit per slide and 1 section per slide of all other organs sampled. One already embedded block of formalin

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doi:10.1016/j.fsi.2009.01.006

fixed, normal human skin (provided by a local hospital) was used as a positive control tissue.

Immunohistochemical staining was done using the avidin–biotin complex (ABC) technique. Sections were deparaffinized in xylene and incubated in 0.3% hydrogen peroxide in 100% methanol for 30 min. Rehydration of sections was done through a graded series of ethanols into distilled water. Antigen retrieval was performed by incubation of sections in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20) pH 6.0 for 40 min at 100 °C. Sections were then allowed to cool for 10 min, then rinsed and washed in PBS (10 min) and incubated in 1% normal rabbit serum for 1 h followed by application of the primary antibody. A commercially available primary antibody was used against human Langerin/CD207 (R&D Systems, Minneapolis, MN, USA). The goat anti-human Langerin/CD207 antibody was applied at a concentration of 4 μg/ml (1:50 from stock solution) to the fish organs and at a concentration of 0.4 μg/ml (1:500 from stock solution) to human skin and incubated overnight at room temperature in a humid chamber; the positive control for Langerin was normal human skin. A negative control included an affinity purified normal goat IgG (Vector Laboratories, Burlingame, CA, USA) which was diluted to the same concentration as the primary antibody. For Atlantic salmon and rainbow trout, spleens from 12 and 6 different fish were checked for Langerin reactivity respectively. Additionally the other noted organs were examined from 4 Atlantic salmon. After overnight incubation the samples were rinsed and washed in PBS (10 min). The secondary biotinylated antibody was a rabbit anti-goat IgG (Vector Laboratories), diluted 1:200 in PBS, applied and incubated in a humid chamber at room temperature for 1 h. After rinsing and washing in PBS (10 min) the sections were incubated in VECTASTAIN® *Elite* ABC reagent (Vector Laboratories) with 0.1% Tween 20 for 1 h, rinsed and washed in PBS (10 min) and stained in a solution of 3,3′-diaminobenzidine tetrahydrochloride (DAB), $NiCl_2$, and H_2O_2 for 10 min. Samples were then washed in tap water (2×10 min) and dehydrated through a graded series of ethanols to xylene. The slides were viewed and photographed with a Zeiss Axiocam system.

To demonstrate Birbeck granules in Atlantic salmon spleen samples were fixed in 2% phosphate buffered glutaraldehyde and stored at 4 °C overnight. The tissue was further processed as previously described by Lovy et al. [6] and embedded into Spurr's resin (Canemco and Marivac, Quebec, Canada). Ultrathin sections (90 nm) were cut, retrieved onto copper super grids (200 mesh) and contrasted with uranyl acetate and Sato's lead. The sections were examined and photographed with a Hitachi 7500 transmission electron microscope operated at 80 kV.

The Atlantic salmon and rainbow trout spleens incubated in Langerin/CD207 showed a population of cells which were positive for Langerin. These cells had a strong cytoplasmic reaction, which was absent in the negative controls treated with affinity purified normal goat IgG (Fig. 1). In total all spleens examined, 12 individual Atlantic salmon spleens and 6 rainbow trout spleens were all positive for Langerin. The Langerin+ cells were found throughout the tissue and in Atlantic salmon the cells were frequently near the periphery of the tissue. Head kidney in Atlantic salmon was also positive for Langerin, although in relatively fewer cells than observed in the spleen. All other organs including the thymus, skin, trunk kidney, gills, GI tract, liver, and heart were negative for

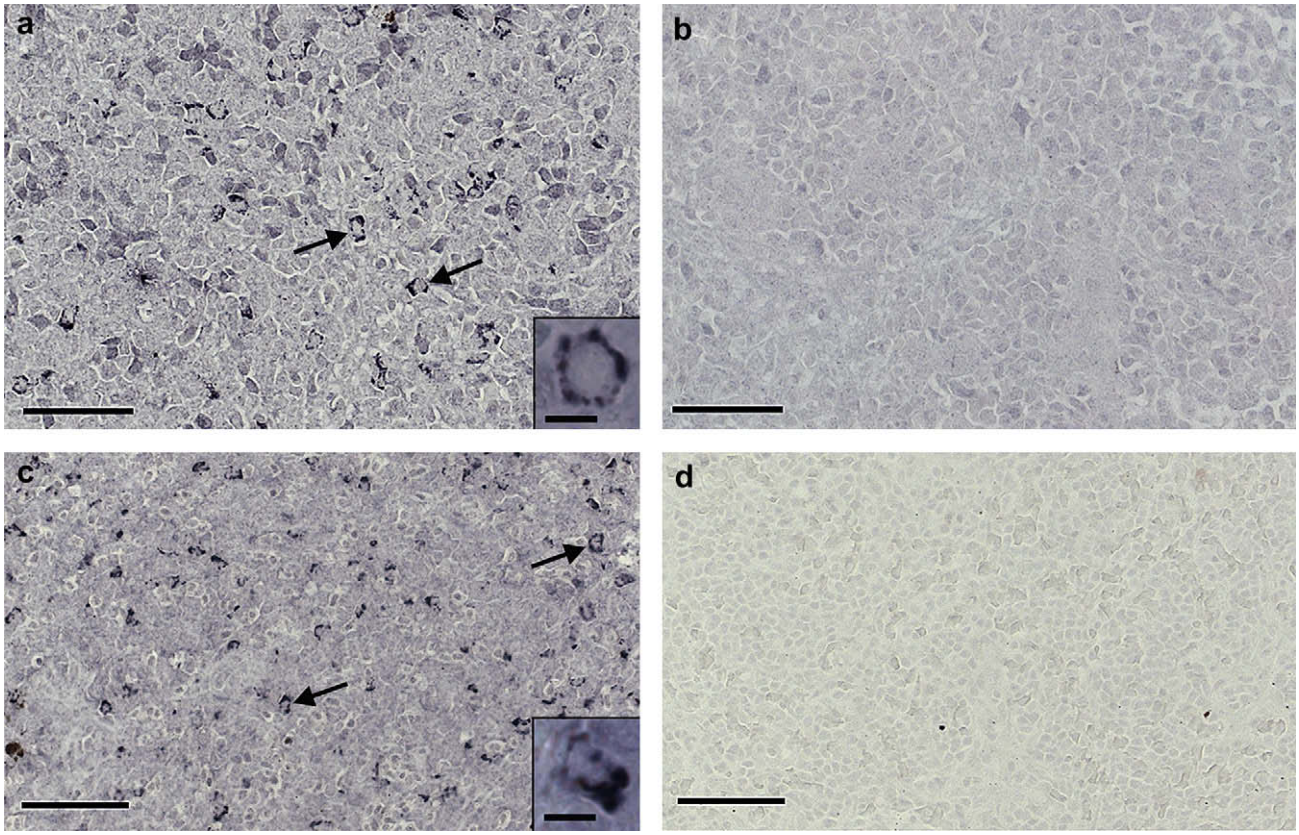

Fig. 1. Langerin/CD207 positive cells in the spleens of salmonids; reactivity is observed in the cytoplasm of these cells (arrows). Bar = 50 μm. (a) Atlantic salmon spleen treated with goat anti-human Langerin/CD207. (b) Atlantic salmon negative control treated with affinity purified normal goat IgG. (c) Rainbow trout spleen treated with goat anti-human Langerin/CD207. (d) Rainbow trout negative control treated with affinity purified normal goat IgG. Insets in (a) and (c) demonstrate the cytoplasmic reactivity of the antibody characterized by dark staining in the cytoplasm and pale nuclear profiles. Bars = 5 μm.