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Histochemistry of Six Lectins in the Tissues of the Flat Fish *Paralichthys olivaceus*

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Abstract

Lectins are glycoproteins that specifically bind carbohydrate structures and may participate in the biodefense mechanisms of fish. In this study, the binding of three lectins, *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Bandeiraea simplicifolia* BS-1 (isolectin B4), *Triticum vulgaris* (WGA), *Arachis hypogaea* (PNA) and *Ulex europaeus* (UEA-I) were studied in the gill, liver, intestine, kidney, heart, and spleen of the flat fish *Paralichthys olivaceus*. DBA was detected in intestinal mucous cells, as well as in gill epithelial and mucous cells. It was weakly detected in renal tubule epithelial cells and in bile duct epithelial cells. The strong SBA staining was seen in the intestinal club cells, in bile duct epithelial cells and renal tubule epithelial cells. There were intense positive reactions for isolectin B4 in gill epithelial and mucous cells, and the strong isolectin B4 staining was seen in epithelial cells of the bile duct and intestine. The strong WGA staining was seen in the gill mucosal cells, sinusoid, renal tubule epithelial cells and mucosal cells of the intestine. UEA-I was detected in the gill epithelial and mucosal cells, bile duct epithelial cells and renal tubular epithelial cells.

These results suggest that the six lectins examined were localized in the covering epithelia of the various organs of the flat fish and they may participate in the biodefense mechanism of the intra body surface in which is exposed to various antigens.

Key words : lectin, flat fish, DBA, SBA, isolectin B4, WGA, PNA, UEA-I.

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Introduction

Lectins are carbohydrate-binding proteins of nonimmune origin that are widely distributed in nature [11]. Lectin binding to the carbohydrate moieties of glycoproteins and glycolipids is important for a variety of biological processes, including cellular adhesion [14], cellular recognition [12], protein folding [7] and signal transduction [3, 10]. All lectin molecules possess two or more carbohydrate-binding sites, a property that is essential for their ability to agglutinate cells or react with complex carbohydrates [22]. Each lectin binds to a specific sugar or group of sugars [20]. Many lectins have been characterized, including peanut agglutinin, wheat germ agglutinin, phytochemagglutinin E, concanavalin A, *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA) and *Bandeiraea simplicifolia* BS-1 (isolectin B4) [6, 21].

In a previous study, it was shown that SBA is a member of the legume family of plant lectins, which have important biological properties, including the induction of mitogenicity in lymphocytes [18]. DBA has carbohydrate specificity for a-linked N-acetylgalactosamine, and DBA-binding targets are expressed specifically in the epithelium of various embryonic mouse organs [4, 17]. The plant lectin *Bandeiraea simplicifolia* (isolectin B4) has a high affinity for a-D-galactose residues in the carbohydrate chains of membrane-associated glycoconjugates [5, 8]. Isolectin B4 was also found in numerous phagocytic macrophages and reactive microglia in and around tumors, which had a similar distribution to that of quinolinate immunoreactive cells [13] and vasoformative endothelial cells from the rat aorta [16]. Mucus is an indispensable chemical barrier in the fish immune system. It contains diverse molecules that may participate in innate or acquired immunity. Among others, lectin and lectin-like molecules are found in the cutaneous mucus of many fish species [1]. It is generally accepted that fish cutaneous lectin contributes to the biodefense system on the body surface because of its agglutinating activity [15].

The flat fish *Paralichthys olivaceus* is important for the Jeju fish culture industry. To improve culture conditions, it is useful to understand biodefense systems in culture fish. Little is known about the distribution pattern of lectins, which are known to be one of various molecules involved in

fish biodefense.

The aim of this study was to localize six lectins including DBA, SBA, isolectin B4, WGA, PNA and UEA-I, in the various tissues of the flat fish *Paralichthys olivaceus*, and to postulate their functional role in the biodefense mechanism of this fish.

Materials and methods

2.1. Fish

Flat fish, *Paralichthys olivaceus*, weighing 100 to 200 g were kept in a flow-through seawater system at ambient temperature (mean 20 ± 2℃) for 5 months in the Jeju Province Fisheries Resources Research Institute. Fish were fed twice daily, and weighed 300 to 400 g at the time of sacrifice (n=5).

2.2. Sampling procedure

Following sacrifice, the liver, kidney, gill, gastrointestinal tract, heart and spleen of each fish were fixed in 10% buffered formalin for 48 hr to prepare for histological examination.

2.3. Histological examination

Specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 ㎛, and stained with hematoxylin and eosin using routine histological techniques. All paraffin-embedded tissue sections from normal fish were stained for the lectin study.

2.4. Lectins

The lectins used in this study were *Bandeiraea simplicifolia* agglutinin (peroxidase-labeled isolectin B4, Sigma, St. Louis, MO), *Dolichos biflorus* agglutinin (peroxidase-labeled DBA, Sigma), glycine max agglutinin (peroxidase-labeled SBA, Sigma), *Triticum vulgaris* agglutinin (peroxidase-labeled WGA, Sigma), *Arachis hypogaea* agglutinin (peroxidase-labeled PNA, Sigma) and *Ulex europaeus* agglutinin I (peroxidase-labled UEA-I, Sigma). The specificity of lectins is summarized in Table 1.

2.5. Histochemistry

Tissues were dehydrated by immersion in a graded ethanol series (70, 80, 90, 95 and 100%), cleared in xylene, embedded in paraffin wax and sectioned at 5 ㎛ on a microtome. The sections were mounted on glass microscope slides, the wax was removed, and sections were hydrated. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. After three washes with PBS, the sections were exposed to 10% normal horse serum, and then incubated with DBA-peroxidase (diluted 1:10), SBA-peroxidase (diluted 1:400), isolectin B4-peroxidase (diluted 1:50), WGA-peroxidase (diluted 1: 20), PNA-peroxidase (diluted 1: 10) or UEA-I-peroxidase (diluted 1: 10) for 3 hrs at room temperature. Peroxidase was developed with diaminobenzidine (DAB)-hydrogen peroxidase solution (0.001% 3,3′diaminbenzidine and 0.01% hydrogen peroxidase in 0.05M Tris buffer). The sections were counterstained with hematoxylin before being mounted.

Results

Histological examination showed that all tissues, including gill, liver, gastrointestinal track, kidney, heart and spleen, were devoid of inflammatory cells, and all tissues were used for lectin histochemistry.

In the gill, DBA (Fig. 1A), SBA (Fig. 1B) and isolectin B4 (Fig. 1C) were specifically detected in the epithelial cells of the gill filament. The covering cells of the gill were largely positive for isolectin B4 (Fig. 1C). WGA (Fig. 1 D) and UEA-I (Fig. 1 F) were detected in mucus and epithelial cells, while PNA (Fig. 1 E) was localized in few epithelial cells of the gill filament.

DBA, SBA and isolectin B4 were weekly detected the gill racker (Fig. 2A, B and C). In the mucosal epithelium, WGA and UEA-I were strongly detected in the mucosal cells (arrow head) and gill racker (arrow) (Fig. 2D and F). But PNA was not detected in all cells (Fig. 2E).

In the skin, a few mucous cells were positive for isolectin B4, but no other lectins were detected.

Table 1. Lectin specificity

Lectin	Abbreviation	Binding specificity
N-acetylgalactosamine group		
Bandeiraea simplicifolia lectin	isolectin B4	α GalNAc, α Gal
Dolichos biflorus agglutinin	DBA	α GalNAc
Glycine max (soybean agglutinin)	SBA	α GalNAc
N-acetylglucosamine group		
Triticum vulgaris (wheat germ)	WGA	β GlcNAc
Galactose group		
Arachis hypogaea (peanut)	PNA	β Gal
Fucose group		
Ulex europaeus-I	UEA-I	α Fuc

In the liver, some bile duct epithelial cells were intensely positive for SBA (Fig. 3B), while only a few were positive for isolectin B4 (Fig. 3C). DBA (Fig. 3A) and PNA (Fig. 3E), however, was not detected in any liver cells. WGA was detected in the sinusoid (Fig. 3D), and UEA-I(Fig. 3F) expression was similar to detection of SBA. There was no lectin reaction in the hepatocytes.

In the esophagus, only isolectin B4 was detected in a few mucous cells and epithelial cells. No lectins were detected in the stomach.

In the intestine, DBA was detected in the intestinal gland mucous cells (Fig. 4A). SBA was expressed in the club cells (Fig. 4B), while isolectin B4 was detected in the epithelial cells (Fig. 4C). The club cells had round heads with a large secretory vacuole situated either above or below the nucleus. WGA was detected in the club cells (Fig. 4D), while UEA-I was detected in the brush border (Fig. 4F). But, PNA was not expressed in the intestine cells (Fig. 4E).

In the kidney (Fig. 5), five lectins were detected in renal tubule epithelial cells, although DBA (Fig. 5A) was less abundant than the other four lectins and PNA (Fig. 5E) was not detected. No lectin reaction was detected in the heart or spleen (data not shown). The histochemical reaction of the six lectins in the gill, liver, skin, gastrointestinal tract and kidney are summarized in Table 2.

Discussion

This is the first study to examine the binding of the 6 lectins including DBA, SBA, isolectin B4, WGA (D), PNA (E) and UEA-I (F) in the cultured flat fish. Both SBA and isolectin B4 showed consistent binding in gastrointestinal epithelial cells, while DBA was limited to epithelial cells in the gill. These findings imply that some lectins preferentially bind to mucosal epithelial cells that may be in contact with various antigens. These findings are in part consistent with those of a previous study, which confirmed that gelectin is strongly expressed in the club cells of the gill, upper alimentary canal, and skin, and postulated that congerin (β-galactoside binding lectin) participates in the biodefense against pathogenic microorganisms and parasites [15].

The physiological and immunological importance of lectins in fish slime has not been sufficiently clarified. Gelatins, one of the major families of animal lectins, exist in a wide range of animal tissue and seem to participate in diverse biological processes, such as immune response modulation [19] and

Table 2. Histochemical localization of *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Bandeiraea simplicifolia* BS-1 (isolectin B4), *Triticum vulgaris* (WGA), *Arachis hypogaea* (PNA) and *Ulex europaeus* (UEA-I)-positive cells in the tissues of normal *Paralichthys olivaceus*

Tissue	Positive cells	Lectin					
		DBA	SBA	Isolectin B4	WGA	PNA	UEA-I
Gill	Epithelial cells	+	+	++	+	+	+
	Mucous cells	+	+	++	++	−	++
	Gill racker	+	+	+	+	−	+
Liver	Epithelial cells of the bile duct	−	++	+	+	−	+
	Hepatocyte	−	−	−	−	−	−
	Sinusoid	−	−	−	++	−	−
Esophagus	Epithelial cells	−	−	+	−	−	−
	Mucous cells	−	−	+	−	−	−
Stomach	Epithelial cells	−	−	−	−	−	−
	Mucous cells	−	−	−	−	−	−
Skin	Mucous cells	−	−	+	−	−	−
Intestine	Epithelial cells	+	+	++	+	−	−
	Mucous cells	++	−	−	++	−	−
	Club cells	−	++	−	−	−	−
	Brush border	−	−	−	−	−	++
Kidney	Epithelial cells of renal tubule	+	++	++	++	−	++
	Glomerulus	−	−	−	−−	−	−
Heart	Cardiac muscle	−	−	−	−	−	−
Spleen	Lymphoid cells	−	−	−	−	−	−

− No binding, + weak binding, ++ intense binding. (Andreas G. *et al.*, 2000)

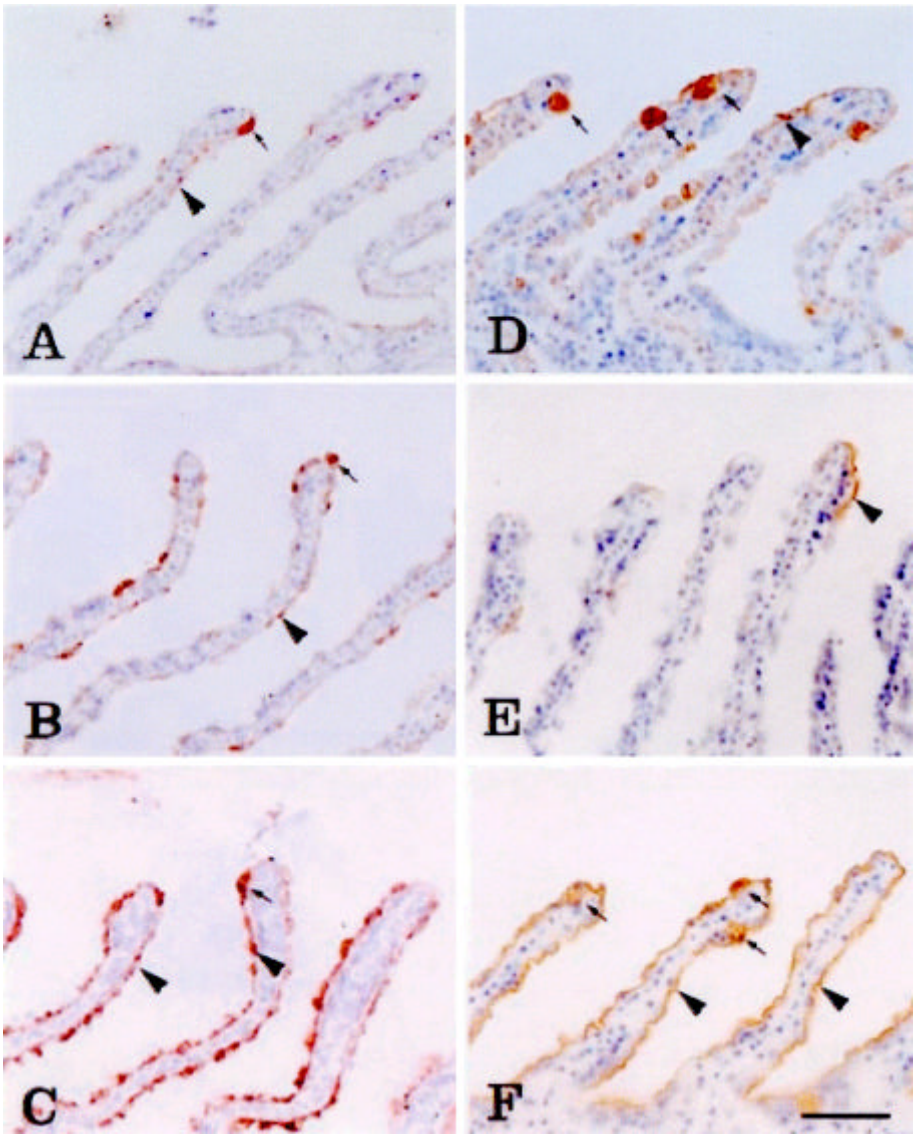

Fig. 1. Histochemical staining of DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA-I(F) in the gill filament of the flat fish, *Paralichthys olivaceus*. Lectins including DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA(F) were specifically expressed in the mucus (arrow) and epithelial (arrowhead) cells of the gill. Counterstaining with hematoxylin. Scale bar = 30 ㎛.

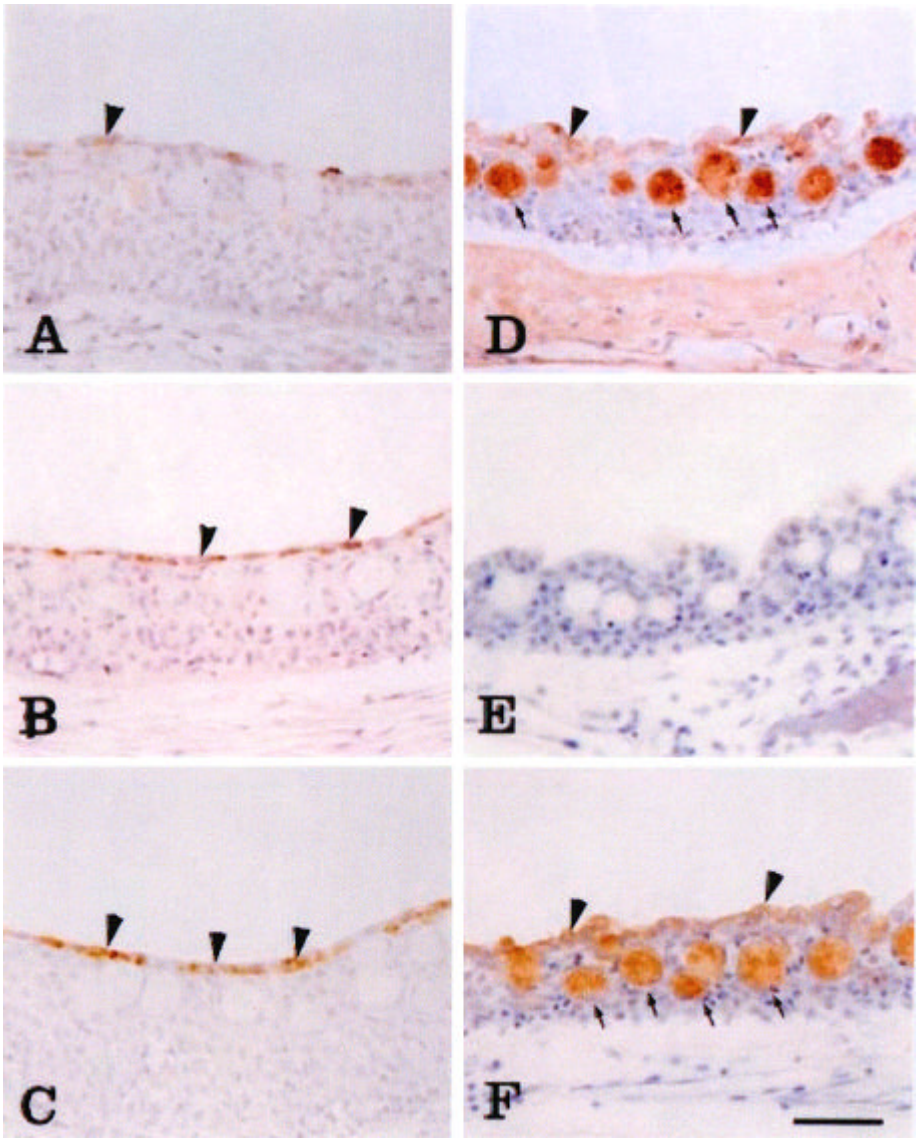

Fig. 2. Histochemical staining of DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA-I(F) in the gill arch of the flat fish, *Paralichthys olivaceus*. Lectins, DBA (A), SBA (B), isolectin B4 (C), WGA(D) and UEA(F) were specifically expressed in the mucosal (arrow) and gill racker (arrowhead) cells of the gill. PNA(E) was not expressed. Counterstaining with hematoxylin. Scale bar = 30 μm.

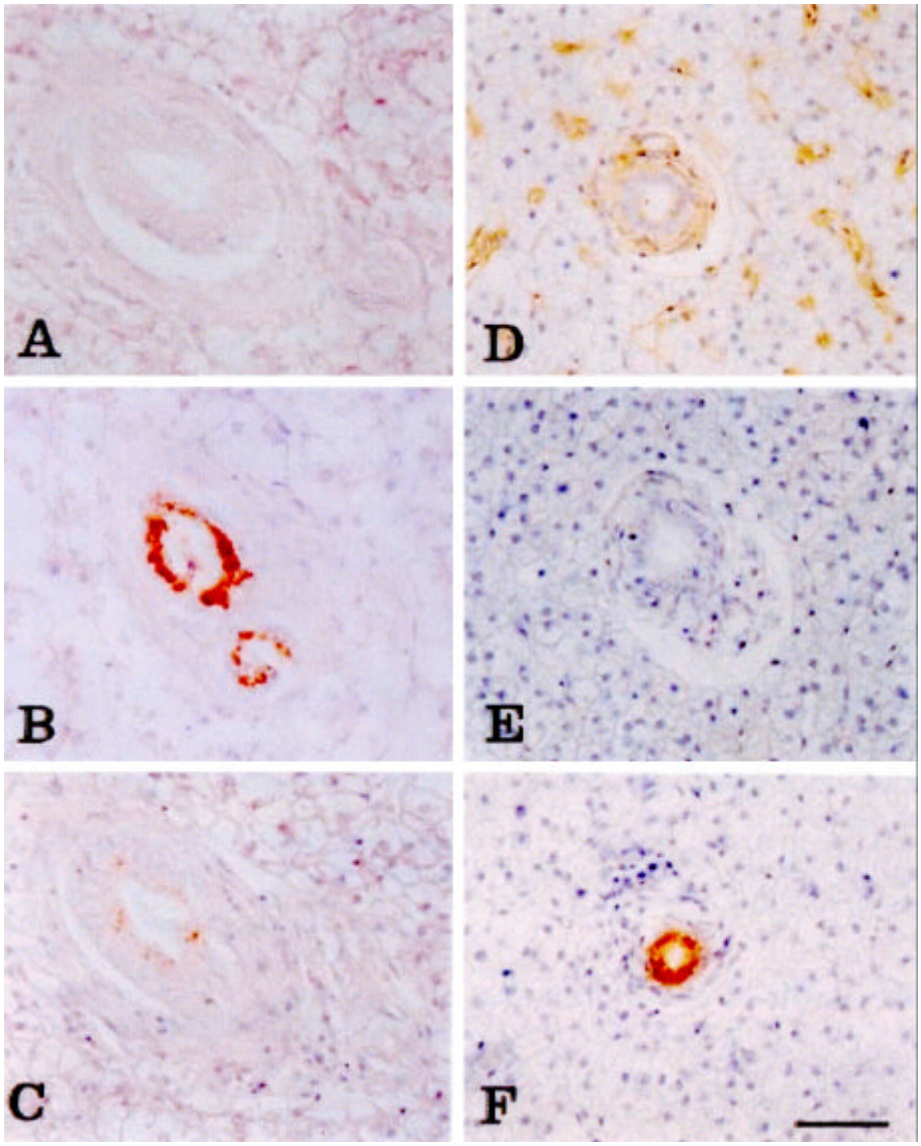

Fig. 3. Histochemical staining of DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA-I(F) in the liver. Some epithelial cells of the bile duct were positive for SBA (B), isolectin B4 (C) and UEA-I(F), but DBA(A) and PNA(E) was not seen in the liver. WGA(D) was expressed in the sinusoid. Counterstaining with hematoxylin. Scale bar = 30 ㎛.

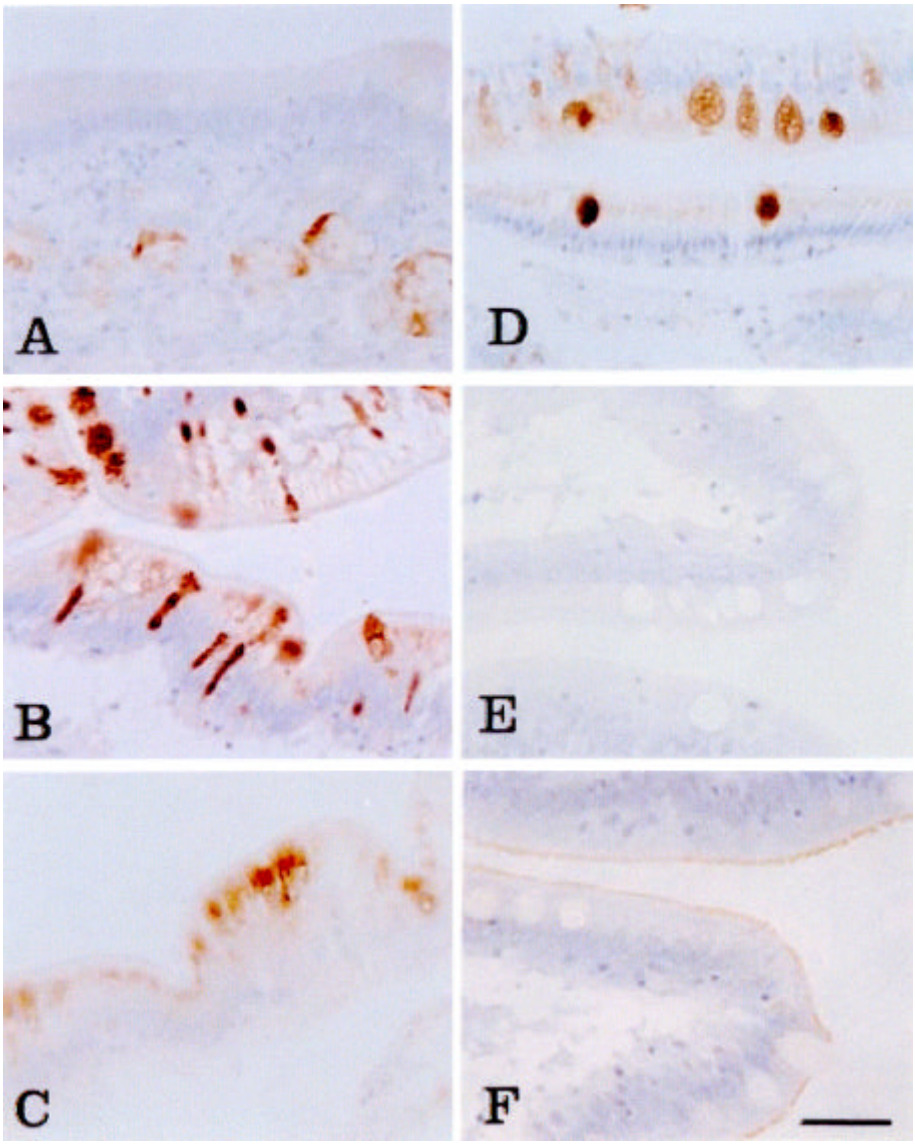

Fig. 4. Histochemical staining of DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA-I(F) in the intestine. DBA (A), isolectin B4 (C) and WGA(D) were expressed in mucous cells (A), while SBA was expressed in club cells (B) and UEA-I(F) was expressed in brush border. PNA(E) was not expressed. Counterstaining with hematoxylin. Scale bar = 30 ㎛.

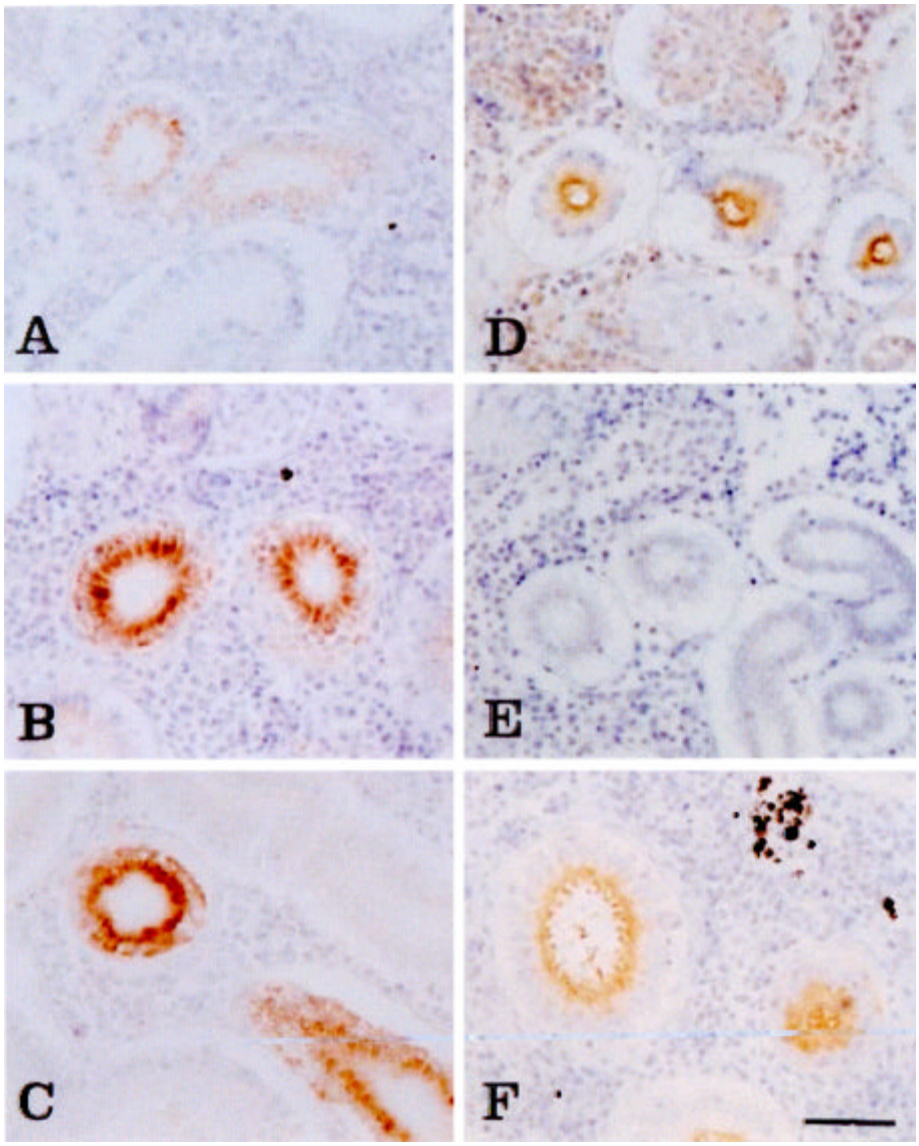

Fig. 5. Histochemical staining of DBA (A), SBA (B), isolectin B4 (C), WGA(D), PNA(E) and UEA-I(F) in the kidney. SBA (B), isolectin B4 (C), WGA(D) and UEA-I(F) were detected in renal tubule epithelial cells. PNA(E) was not expressed. Counterstaining with hematoxylin. Scale bar = 30 ㎛

cell growth regulation [23]. Protection from pathogens may be a potential role for fish mucosal lectin and epithelial lectin, although that role was not examined in this study.

It is generally assumed that fish mucous lectins are involved in innate immunity against microorganisms. Studies have suggested that in some fishes, the mucosal agglutinins are sufficient for excluding bacteria from the skin [9].

The six lectins examined in the present study are also expressed in cell groups that are involved in mucus secretion in the epithelia of various organs. Based on the distribution of these lectins, DBA, SBA, isolectin B4, WGA, PNA and UEA-I may also have a biodefensive role in flat fish.

These results show that lectins are preferentially restricted to the epithelial cells of the covering mucosa in cultured flat fish, and suggest that they are involved in the biodefense mechanism of fish. Further study is needed to confirm their exact role during pathophysiological conditions such as bacterial infection.

Acknowledgements

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