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Lectin histochemistry shows the comparative biosynthesis and cellular biodistribution of alpha L-fucose residues in some tissues of tetrapoda representatives

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ABSTRACT

Fucose is a monosaccharide that plays several immunological roles. This study investigated the comparative biosynthesis and cellular biodistribution of fucose residues in some tissues of tetrapoda representatives using lectin histochemistry. In this study, the mouse was used as a representative for mammalian, pigeon for avian, lizard for reptilian, and toad for amphibians. The localization of the fucose residues was seen in several cell types of mice ileum, such as villi microfold (M) cells, goblet cells, some of intestinal crypts cells, and lamina propria cells. In other tetrapoda representatives, fucose was only seen in M cells of lizard ileum and some cells of villi lamina propria of pigeon, lizard, and toad. It was also observed in the pancreatic acinar cells of the mouse and some cell aggregations of pancreatic parenchyma of the lizard. Contrarily, it was not seen either in pigeon or in toad pancreases parenchyma. Spleen of all animals showed the fucose residues in some splenic cells in the red pulp only, barring the white pulp. The liver parenchyma of all tetrapoda representatives hadn't fucose residues. The fucose cellular biodistribution in some cells of tetrapoda representatives differed based on the cell type. In the mouse, it was highly seen in the apical cytoplasm of the villi M cells as well as in the cup-like part of goblet cells. In addition, it was seen as "rings" in the granule membranes of the Ulex europeaus agglutinin I ($UEAI^+$) cells in the intestinal crypts cells. Furthermore, the $UEAI^+$ cells in the lamina propria showed fucose granules in their cytoplasm. There is no clear evidence about the relation between the cellular biosynthesis of fucose residues and mucosal immune cells. The role of fucose residues in the pancreatic acinar cells are not well understood and need further investigations. In this study, fucose residues were synthesized in several types of cells in the mouse ileum, spleen and pancreas as compared with other tetrapoda. The data obtained from this study can help us to get more information about the cellular biodistribution and synthesis of fucose residues in several animal species rather than mammalians.

Introduction

Fucose residues are hexose deoxy sugars and constitute several cell structures. Fucose residues were predominantly synthesized in the immune cells of mouse ileum (Awaad et al., 2012a, 2012b; Gringhuis et al., 2014). They play a pivotal immunological role in the interactions between the host and pathogens, as well as the adhesion of leukocyte and endothelial cells (Becker and Lowe, 2003). Fucose residues can be localized in several cell-surface oligosaccharides involved in adhesion and cell recognition, such as goblet cells, intestinal microfold (M) cells, and dendritic cells (DCs) (Awaad et al., 2012a, 2012b; Awaad, 2014; Gringhuis et al., 2014). Previously in vitro study showed that epithelial cell surface had sulfated fucose residues serve as adhesive ligands for CD11b/CD18 during the neutrophils transmigration through the mucosal surface (Zen et al., 2002). In another study when α-L-fucose residues were blocked on the tumor cell membrane the macrophages were no longer able to respond to lipopolysaccharide (Cameron, 1985). Additionally, the immunological activity of pathogens with intestinal mucosa produce mucins containing fucose in the intestinal lumen as an immune response against pathogens (Chow and Lee, 2008). The gut commensal Bacteroides thetaiotaomicron could induce the rat mucosal mucin to produce fucose residues for its own metabolism (Enss et al., 1996). Also, Gebert and Cetin (1998) revealed that mouse intestine react moderately with UEAI in the mucous granules of the goblet cells and brush border of the enterocytes only.

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The localization and biodistribution of fucose residues in tissues and cells is usually investigated with specific binding lectins. The most popular fucose-binding lectins include the Ulex europaeus agglutinin I (UEAI) (Awaad et al., 2012a, 2012b), Lotus tetragonolobus (LTL) (Holthöfer et al., 1982), Aleuria aurantia (AA) (Matsumura et al., 2007). Previously, the biosynthesis of fucose residues was not investigated in detail. This is especially true for the lower vertebrates, such as avian, reptilian, and amphibian animals. In mammalian species, it was reported that fucose residues were synthesized in several tissues of some species, such as the olfactory sensory neurons of immature mice (Ducray et al., 1999), the endothelial cells of humans (Kirkeby et al., 1993), and the olfactory mucosa supporting cells and the Bowman's glands of Korean roe dear (Park et al., 2014). Furthermore, I previously reported the biosynthesis and induction of fucose residues in the Peyer's patches (Awaad et al., 2012a, 2012b), cecal patches, spleen (Awaad, 2014), and female genital tract (Awaad, 2015) after nanoparticles oral administration. In addition to the M cells in the Peyer's patches, the M cells of the intestinal villi in the mice also showed fucose residues in the apical cytoplasm (Jang et al., 2004; Mabbott et al., 2013). Previous literatures did not reveal enough data about the biosynthesis of fucose residues in avian animals (Honas et al., 2009) and reported poor data in the reptilian (Fuenzalida et al., 2000; Labate et al., 1997) and amphibian animals (Farias et al., 2006; Sancar et al., 2009; Villalba et al., 1993). In an earlier study, pigeons were shown to have higher levels of L-fucose in the brain, heart, lung, and muscle homogenates compared with those found in the spleen and liver tissue homogenates (Honas et al., 2009). Furthermore, fucose binding lectins such as LTL and AA react strongly with intestinal mucin of Pigeon fanciers' lung rather than UEAI for fucose specific lectin (Baldwin et al., 1999).

In the reptilians, some researchers reported that the L-fucose residues were densely appeared in the cell surface and cytoplasm of multinucleated odontoblast cells but not in the mononuclear odontoblast cells during the lizard's (*Liolaemus gravenhorsti*) tooth development (Fuenzalida et al., 2000). Further, during reproductive periods, fucose residues were observed on the surface of ciliated and nonciliated cells found in the efferent ducts of lizards (*Podarcis siculus campestris de Betta*) (Labate et al., 1997). It was also found in the fibrous sheath of the spermatozoa flagella in lizards (*Tropidurus itambere*) (Ferreira and Dolder, 2003). As for amphibians, fucose residues were previously detected in the zona pellucida of Bidderian and ovarian follicles in the Brazilian toad (*Bufo ictericus*) (Farias et al., 2006). In another report, fucose residues were seen in the cytoplasm of flask cells and flask-shaped secretory cells in the dermal glands and the epidermal glandular ductal cells in the skin of toads (*Rana perezi*) (Villalba et al., 1993). Fucose production depends on the cell type; fucose was only synthesized in the eosinophilic cells of the gastric mucosa but not in the goblet cells or brush border of small intestine in the frog (*Rana ridibunda*) (Sancar et al., 2009).

Therefore, it is crucial to investigate the biosynthesis of fucose residues in the lower vertebrates comparatively in order to understand their important function in each tetrapoda class. The function of fucose residues and their cellular biodistribution in mammals' tissues needs more investigation to illustrate their biological role. The lower vertebrates have lower-developed immunological systems compared with mammalian animals. Therefore, it is important to understand the developmental localization and biodistribution of fucose residues in some tissues of tetrapoda representatives related to each class. I used one species as a representative animal of each tetrapoda class in this study. In this study I assumed that fucose residues biosynthesis had relationship to some immune cell functions. It is well known that ileum and spleen contains several immune cells as compared with pancreas and liver. These selected organs also are easy to obtain from the tetrapoda representatives. Therefore, using lectin histochemical techniques, I investigated the biosynthesis and cellular biodistribution of the fucose residues specific to UEAI lectin in the ileum, spleen, pancreas, and liver of some tetrapoda representatives. The cellular biodistribution of these molecules in mammals will be compared with those in other tetrapoda species.

Materials and methods

Ulex europaeus agglutinin I (UEA I), conjugated with fluorescein isothiocyanate (FITC), and 4′,6-diamidino-2-phenylindole (DAPI) fluorescent dye were purchased from Sigma-Aldrich Chemical Co., UK.

Animals

As shown in Table 1, different numbers of adult male representatives of each class of tetrapoda were collected for this experiment from different sources located in Sohag Governorate, Egypt. To verify their ideal conditions, the animals were collected during the summer (May–August) of 2013. I used the most popular animals in each class as they were readily available and are commonly used for undergraduate research. In this study, the mouse (*Mus musculus*) served as a representative of the mammalian class, the pigeon (*Columba livia domestica*) as one of the avian class, the lizard (*Trachylepis vittata*) as one of the reptilian class, and the toad (*Bufo bufo regularis*) as one of the amphibian class. The animals were kept separately in separate cages in the animal house under normal conditions, namely, a 12:12-h light:dark cycle at 25 °C room temperature with free access to water and food. They were observed two weeks before the date of the experiment. The abnormal animals were excluded from the experiment. Three healthy animals from each class were selected and used in the experiment. All experiments were carried out according to the Guidelines for the Care and Use of Animals, approved by the Animal Experiments Committee in Sohag University.

Lectin histochemistry

All the animals were anesthetized with chloroform in a glass cage. The anesthetized animals were fixed dorso-ventrally on the dissecting tray and the abdominal skin and peritoneal membrane were opened and removed. Some tissues, such as the ileum, spleen, pancreas and liver were removed, cut into small pieces and fixed in 4% paraformaldehyde for cryosections. The number of tissues obtained for each class/species was associated with the number of animals collected as shown in Table 1. The sex and puberty of all animals was detected during their dissection. The selected tissues

Table 1
General information about the tetrapoda representative animals collected from Sohag Governorate, Egypt.

Class	Scientific name	English name	Sex	Weigh/gm	Number	Source
Mammalian	*Mus musculus*	Mice	Male	32	5	Sohag University Animal House
Avian	*Columba livia domestica*	Pigeon	Male	345	4	Sohag Governorate, Naser city
Reptilian	*Trachylepis vittata*	Lizard	Male	18	4	Sohag Governorate, Akhmim city
Amphibian	*Bufo bufo regularis*	Toad	Male	35	6	Sohag Governorate, Akhmim city