Histological detection of lectin binding sites in human gastrointestinal mucosa[1]

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Summary. Four lectin-peroxidase conjugates (DBA, PNA, BSA I, UEA I) were used to stain intracellular carbohydrates in normal mucosa of human stomach and duodenum. Heterogenous patterns were observed with each of the lectins in a given cell population.

Numerous studies on the morphology of normal human gastric and duodenal mucosae exist, but although sophisticated histochemical methods have been employed, changes in the cells are still subject to controversy[2-7]. A useful approach is offered by studies of lectin binding to tissue sections. Due to their high affinity and specificity for sugar residues, lectins have become important tools for biochemical and histological characterization of cellular carbohydrates[8-11]. For the latter, lectins conjugated e.g. with peroxidase are used. Histological studies help to elucidate carbohydrate composition in glycoconjugates of gastrointestinal cells and may fill a gap between biochemical analysis and classical histopathology. Thus, it is expected that detailed information can be gained about cell function in the normal state and in disease.

Materials and methods. Samples of human gastric and duodenal mucosae of blood groups A, B, AB and 0 were taken during surgery and processed for histology as described[12,13]. Peroxidase (HRP) conjugates of *Arachis hypogaea* (PNA), *Dolichos biflorus* (DBA), *Bandeiraea simplicifolia* (BSA I) and *Ulex europaeus* (UEA I) agglutinins were obtained from Medac GmbH (Hamburg, FRG).

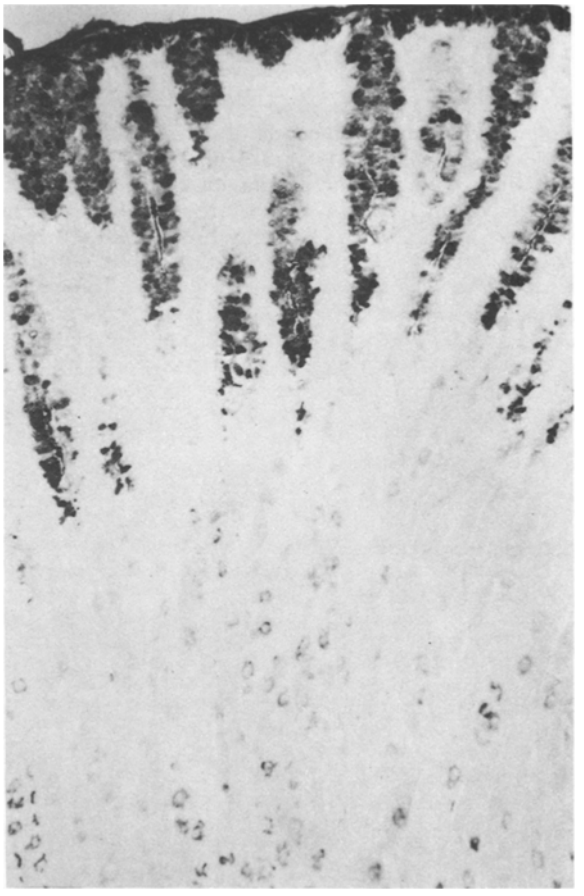
Lectin binding studies were performed on 5-7-µm-thick sections analogous to those for which immunohistological procedures are described[12,13]. Briefly, endogenous peroxidases were inhibited[12] and the sections were incubated in lectin-HRP (0.001-0.02 mg/ml PBS) for 24 h at 4 °C. Unreacted conjugates were removed by 3 successive washings in PBS for 5 min each. Peroxidase activity was demonstrated as described[14]. Control reactions consisted of incubation of parallel sections in HRP labeled lectin solutions containing their respective inhibitors: 0.2 M lactose for PNA; 0.2 M D-galactose for DBA and BSA I; 0.2 M L-fucose for UEA I.

Results and discussion. Normal 'secretor' type mucosae are known to express blood group A, B or 0 (H) antigens in surface epithelium and gastric pits[15], and one would expect that PNA, BSA I and UEA I detect blood groups A, B and 0 (H), respectively. However, histological staining patterns did not reflect localization of blood groups exclusively because lectin bindings were found equally in stomachs from donors with blood groups A, B, AB and 0 (H). All observations with the different types of cells and the lectins are summarized in the table; in controls, the respective inhibitors abolished all tissue staining. HRP conjugates of UEA I (specific for α-L-fucose)[16] stained the bulk of surface epithelial, neck and Brunner's gland cells. Chief cells and antral gland cells were mainly positive, too. These results are in agreement with previous reports in which PAS positive neutral mucins of gastric epithelial cells were described as being fucomucins[2,3,6,17], and fucose was suggested to be a possible terminal sugar in epithelial mucins[18]. Incubation of mucosa cells with PNA-HRP (accessible galactosyl residues[19]) gave a heterogenous picture. This supports the idea that different carbohydrate moieties

Lectin binding to normal gastric and duodenal mucosae

Localization	UEA I	PNA	DBA	BSA I
Surface epith. cells	+[a]	+ and −	Mainly+ Few −	+
Neck cells	+	+ and −	−	−
Parietal cells	−	−	+	+
Chief cells	Mainly+ Few −	Mainly− Few +	−	−
Antral gland cells	Mainly+ Few −	Mainly+ Few −	+ and −	+
Brunner's gland cells	+	Mainly+ Few −	Mainly+ Few −	+
Goblet cells	+ and −	−	+ and −	+
Columnar cells	+	−	Mainly− Few +	+

[a]Reactions: −, no; +, clear positive; + and −, positive as well as negative cell areas.



Histological section of human gastric mucosa stained by DBA-HRP conjugates. Note DBA binding by surface epithelial cells and parietal cells. × 220.

mainly in the terminal position occur in morphologically identical cell types. In the case of intracellular staining of galactose residues we do not know whether the latter represent terminal saccharides or incompletely glycosylated glycoconjugates. The distribution of binding sites for DBA (specific for N-acetyl-galactosamine[20]) and BSA I (specific for D-galactopyranosyl[21]) in gastrointestinal cells showed a lack of correlation between PAS reactivity and lectin binding. While surface epithelium and Brunner's gland cells were mainly positive for DBA, PAS positive neck cells usually failed to stain with DBA-HRP (fig.). Antral gland cells also gave an irregular staining pattern; nonreacting and reacting cells were seen in the same histological preparation. PAS negative parietal cells were positive with DBA; chief cells were almost negative. In the case of BSA I binding, a similar pattern was found. Goblet cells and columnar striated cells exhibited an irregular staining pattern with the 4 lectins used (see table). Generally, the lectins did not bind only to cells with secretory activity but also to glycosylated nonmucus cell products. The heterogeneous lectin binding in histological preparations of normal mucosa suggests qualitative and quantitative differences in the composition of glycoconjugates. This might be due to different functional states and maturation stages; studies including diseased mucosae are needed.

1 This study was supported by the Deutsche Forschungsgemeinschaft (Ku 257/5-2 and Ku 257/6-3) Bonn (FRG).

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0014-4754/83/030286-02$1.50 + 0.20/0

Mast cells in the pars flaccida of the tympanic membrane. A quantitative morphological and biochemical study in the rat[1]

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Summary. The present study has shown that the pars flaccida of the tympanic membrane of the rat is extremely rich in mast cells. The findings were compared with those from earlier investigations in the rat; the pars flaccida is one of the tissues in this animal in wich mast cells are most abundant.

Since its discovery by Ehrlich in 1877, the mast cell has attracted great interest and has been shown to be widely distributed in the connective tissue throughout the body in humans as well as in other species. The mast cell stores pharmacologically potent mediators, which after appropriate stimulation are released, and which participate in various inflammatory and hypersensitivity reactions (see eg. Selye[3], Bloom[4]).

Until lately, mast cells of the middle ear have been exclusively demonstrated in the hypotympanon, mainly in the connective tissue of the mucosal lining close to the tympanal orifice of the Eustachian tube[5]. However, in a recent study on the morphology of the pars flaccida of the tympanic membrane, this membrane portion was found to be extremely rich in mast cells[6]. In the present paper quantitative data will be presented on these pars flaccida mast cells and their possible role in the pathogenesis of a common pathological entity - otitis media with effusion - will be discussed.

Material and methods. 30 male Sprague-Dawley rats, average weight 200 g, were used for the study. They were anesthetized by i.v. injection of sodium pentobarbital. For light microscope studies, a mixture of ethanol-formalin (9:1) was used as fixative and instilled in the external auditory canals of 20 rats. After decapitation their tympanic membranes and, in 6 rats, parts of their mesenteries were carefully dissected out and immediately fixed. All specimens were stained with toluidine blue. Directly after staining, mast cell numbers in individual pars flaccidas were calculated and related to the total area of pars flaccida using a point counting method[7]. Thickness measurements of pars flaccida were performed on semithin sections (1 µm) of glutaraldehyde-fixed and Epon-embedded tissue. For determination of histamine content, 10 anesthetized animals were decapitated and the pars flaccida was dissected out. Its wet weight was determined, and the specimens were then rapidly frozen. Histamine was assayed according to the method of Shore et al.[8].

Results. Mast cells were randomly distributed throughout the pars flaccida (fig. 1) but were confined to a rather narrow layer of the propria immediately below the keratinized squamous epithelium (figs 2 and 3). It should be noted that pars tensa was prepared in the same manner but was totally lacking in mast cells. For comparison the