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Human small intestinal epithelial cells constitutively express the key elements for antigen processing and the production of exosomes

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Abstract

In humans, the small intestinal epithelial cells (IEC) have a high constitutive expression of MHC class II (MHC II), and contains lysosomes. The IEC also contains MHC II rich multivesicular compartments and has been shown to produce exosomes. This suggests a role for the IEC in antigen processing and presentation either directly or indirectly by the production of exosomes. However, the presence and localisation in the IEC of other key molecules involved in this process has not been studied previously.

In the present work, we have investigated small intestinal biopsies from healthy adults and the HT29 IEC cell line with monoclonal antibodies against molecules involved in the antigen processing/presenting systems and molecules typically found on exosomes derived from professional APCs and IECs. Immunohistology was performed to study the expression and localisation of MHC II (HLA-DR), HLA-DM, MHC I (HLA-ABC), CD1d, Invariant chain, Lamp-1, CD68, CD63, B7.1, B7.2, ICAM-1, Cathepsin D/S/L and the IEC specific marker A33 in the IECs.

We found that the IECs from the biopsies constitutively express MHC II, HLA-DM, MHC I, Invariant chain, Lamp-1, CD 68, CD63 and A33, and these markers were also found in the IFN-g treated HT-29 cells. All these molecules were found apically in the IECs of the biopsies, localised mainly in vesicular structures. Interestingly, in the baso-latereral area of the IEC, only MHC II, MHC I, Lamp 1, CD68, CD63 and A33 were found and also here with vesicular staining pattern which matches the molecules previously found on exosomes derived professional APCs and human IEC lines. CD1d, B7, ICAM-1, CD9 and cathepsin D and L were absent in the IEC compartment, but cathepsin S showed a relatively weak staining in the apical part of the IEC.

The staining pattern and the morphological localisation of these markers suggest a prominent antigen processing/loading and trafficking compartment, and a possible baso-lateral release of exosomes in the normal human IEC.

Keywords: MHC class II; Epithelium; Intestine; Human; Immunohistology

Introduction

The intestinal epithelial cell (IEC) has, apart from its properties of absorbing nutrients and transport IgA into the gut lumen [1], been looked upon as a mere passive barrier protecting us from the potentially harmful contents of the intestine. It is however clear that even under homeostatic non-inflammatory conditions a considerable amount of high molecular material from the gut content pass through the epithelium and can be found in the circulation. Thus, several studies have shown that, e.g., undegraded food derived proteins can be found in the circulation shortly after a meal [2,3]. In experimental animals, it has been shown that particularly in the proximal small intestine there is a reflex pinocytotic uptake of luminal content by the IEC as soon as 5 min after feeding a protein-rich solution [4,5]. The IEC has a high constitutive expression of MHC class II (MHC II) molecules in man and rodents and absorbed material has been shown to co-localise with MHC II in the apical part of the IEC [5–7]. These data and the presence of lysosomes

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and multivesicular MHCII enriched structures in the IEC [8] have led to the assumption that a classical late endosomal compartment resembling that found in professional APC is formed in the IEC. This notion was also supported by the early work showing that IEC can present antigen to T cells [9–11]. We have recently described, in a rat model, that IECs both in vivo and vitro release exosome-like structures (tolerosomes) [12] carrying MHC II molecules and that effectively induce tolerance to a fed protein when transferred to naive recipients. The production of these tolerosomes not only requires a complete antigen uptake, processing and loading system within the epithelial cell but also trafficking and homing systems. It has also been shown that human IEC lines produce exosome-like structures in vitro with a polarised expression of some molecular markers [13].

In the present study, we have used monoclonal antibodies against molecules involved in the antigen processing system and found on exosomes derived from professional APCs like DC and B cells [14,15] and examined by immunohistology their expression and localisation in the IECs of duodenal biopsies from healthy adult humans.

The following markers were traced on frozen sections using monoclonal antibodies: MHC II (HLA-DR), HLA-DM, MHC I (HLA-ABC), Invariant chain, Lamp1 CD68, CD63, B7.1, B7.2, ICAM-1 (CD54), CD9, Cathepsin D, Cathepsin S, Cathepsin L, MICA, CD1d, Cytokeratin and the IEC specific marker A33.

We found that the IEC constitutively expressed MHC II, HLA-DM, MHC I, Invariant chain, Lamp1, CD 68 and CD63. All these molecules were found apically in the cell localised in vesicular structures. Interestingly, in the basolatereral area of the IEC, only MHC II, MHC I, Lamp 1, CD68 and CD63 were found and also here with vesicular staining pattern which matches the molecules previously found on exosomes from DCs, B cells and human IEC lines. B7 and ICAM-1 were absent in the IEC compartment.

The staining pattern and the morphological location of these markers strongly suggest a prominent antigen processing/loading and trafficking compartment constitutively present in the normal IEC and also supports the view that exosomes/tolerosomes are produced by and exported from the IEC which under normal circumstances lacks expression of co-stimulatory molecules possibly facilitating their tolerogenic activity.

Materials and methods

Subjects

Six healthy volunteers (3 males and 3 females) with a mean age of 46 years (range 24–68 years), and without either a history of gastrointestinal illness or food or inhalant allergy were subjected to endoscopy and biopsies were collected from the duodenum. The volunteers served as a control group in a previously published study on food allergic patients [16].

Immunohistochemistry

Endoscopic biopsy specimens (3–5 pieces) from the proximal part of the duodenum were taken with a Fujinon EG 200-S P instrument (Fujinon Inc., Wayne, NY and USA). The tissue specimens were immediately embedded in OCT compound (Tissue-Tek Miles. Inc. ELK Hart., IN, USA), and frozen in isopentane (2-metyl-propane), precooled by liquid nitrogen, and finally transferred into pure liquid nitrogen. Biopsies were kept at −70°C, until cut analyzed. Informed consent was obtained from the participants, and the Ethics Committee of the Medical Faculty, University of Gothenburg approved the study protocol.

Cryostat sections (5 μm) were fixed in ice-cold acetone (30 s in 50% acetone followed by 5 min in 100% acetone), and then air-dried for 30 min. Endogenous peroxidase activity was blocked by incubation for 20 min at room temperature in a solution of 1 U/L glucose oxidase (Sigma, St. Louis, MO, USA), 10 mM glucose and 1 mM NaN_3 preheated for 15 min at 37°C. For immunostaining, the slides were incubated overnight in a humid chamber at 4°C with the following mouse monoclonal antibodies to human markers: HLA-DRα (TAL 1B5, Dako), HLA-DM (MapDM1, Pharmingen), invariant chain CD74 (MCA 688, Serotec), CD68 (KP1, Dako), CD63 (H5C6, Pharmingen), LAMP1 (CD107a, KP1, Southern Biotech), CD54 (cat no. 559817, Pharmingen), Cathepsin D (Transduction lab, C47620), Cathepsin L (Transduction lab, C78820), Cathepsin S (DPC-Biermann, D6123, Germany), cytokeratin (Novocastra NCL-50), B7.1 and B7.2 (Pharmingen), CD9 (cat no. 555371, Pharmingen), MICA (SR99, a kind gift from S. Caillat-Zucman Laboratoire d'Immunologie- Equipe AVENIR INSERM Paris, France), CD1d (NOR3.2, Serotec) N and A33 (a kind gift from Dr. J. Heath, Molecular and Cell Biology Laboratory, Ludwig Institute for Cancer Research, Parkville, Victoria, Australia). The antibodies were diluted to an appropriate concentration in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) (Sigma) and 0.05% saponin. Sections used as negative controls were incubated with isotype-matched irrelevant MoAb (mouse IgG1, κ Dako). After washings with PBS (3 × 5 min), the sections were incubated for 1 h at room temperature with a biotinylated F $(ab)_2$ rabbit anti-mouse immunoglobulin (DAKO) diluted 1:400 in PBS + 2% BSA + 2% normal human serum. Thereafter, the slides were incubated with avidin-conjugated peroxidase (ABC-complex, Dako) for 30 min. The peroxidase staining was revealed with amino-ethyl-carbazole (Sigma), followed by a light counterstaining with Mayer's hematoxylin, and mounting in Aquamount (BDH Laboratory, Poole, UK). The microscopical examination was performed using a Leica DMB microscope equipped with a digital colour camera (Leica 300F).