Apo-transferrin is internalized and routed differently from Fe-transferrin by Caco-2 cells: a confocal microscopy study of vesicular transport in intestinal cells

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Caco-2 cells grown as monolayers on porous membranes in bicameral chambers have been used to study the transport of Fe from the apical (lumenal) chamber to the basal (serosal) chamber. The transport of Fe is stimulated by the presence of either apo-transferrin (apo-Tf) or ferri-transferrin (Fe-Tf) in the basal chamber with the stimulation occurring at much lower concentrations of apo-Tf than Fe-Tf. To further explore the involvement of Tf in Fe transport across the basal surface, laser scanning confocal microscopy with 3-dimensional reconstruction of the confocal images was used to visualize the internalization of Texas Red–labeled apo-Tf and Bodipy-labeled Fe-Tf from the basal chamber. These studies show that apo-Tf was readily internalized and routed preferentially to a perinuclear region of the Caco-2 cells while internalized Fe-Tf stayed preferentially below the nuclei. These findings suggest that intestinal cells have a specialized mechanism to recognize and sort apo-Tf. (Blood. 2000;95:721-723)

Introduction

The physiology of iron uptake across the intestinal epithelium has been well described. Three recently described proteins expressed in the intestine — Nramp2,[1,2] HFE,[3] and Haephestin[4]—have enhanced understanding of the mechanisms involved in intestinal iron transport. There is controversy as to whether transferrin (Tf) plays a role in the release of iron from the intestinal epithelium basal surface.

Caco-2 cells grown in bicameral chambers are a model system for intestinal iron transport.[5-9] These cells can internalize apo-transferrin (apo-Tf) as well as ferri-transferrin (Fe-Tf) from the basal chamber into distinct vesicles. Both forms of Tf stimulate iron transport into the basal chamber.[6,10-12] The present studies, using fluorescent labeled apo-Tf and Fe-Tf, investigate by confocal microscopy the internalization of Tf into Caco-2 cells. Apo-Tf was internalized to a perinuclear position and Fe-Tf to a more basal position. The results support a model in which Caco-2 cells distinguish apo-Tf from Fe-Tf and route the two to different compartments, with apo-Tf being routed to a compartment where it could acquire newly adsorbed Fe.

Materials and methods

Cell culture

Caco-2 cells, from American Type Culture Collection #HTB37 (Rockville, MD), were grown in Transwell bicameral chambers as previously described.[5] Confocal microscopy was performed only after the transepithelial electrical resistance had risen to more than 250 Ω/cm^2.[5] The cell monolayers were depleted of serum proteins as previously described.[11]

Fluorochromes and transferrins

Bodipy- and Texas Red–labeled human transferrin and ToPro-1 were obtained from Molecular Probes (Eugene, OR). The Tf was rendered to the iron-free, apo form by lowering the solution pH to 4.5 with HCl in the presence of Chelex resin (30% vol/vol) under constant stirring. The pH of the solution was slowly raised to 7.2 with small aliquots of NaOH. Aliquots of apo-Tf were frozen and used within 6 months. Fe-Tf was formed from the apo-Tf by loading the Tf with Fe to a 95% saturation.[13]

Laser scanning confocal microscopy and image analysis

Images were collected by means of a 60 × Nikon (apo-planar DIC) objective in a Nikon inverted microscope with a BioRad 1024 confocal scan head using the 1024 Sharp image software. Images were collected at 512 × 512 pixel resolution. The cell monolayers were optically sectioned in the z-axis, and the images were collected in a sequential mode to minimize the crossover between channels. When apo-Tf and Fe-Tf were offered simultaneously, the microscope parameters for the collection of images were kept constant between samples to allow comparison of the 2 fluorescent-labeled Tfs. The step size in the z-axis was varied from 0.5 to 1.2 μm to obtain 25 to 30 slides per imaged field. The images were transferred to a Macintosh PC 8100 computer and analyzed with NIH Image 1.60 (available by anonymous FTP from zippy.nimh.nih.gov).

Results and discussion

Laser scanning confocal microscopy of apo-Tf and Fe-Tf internalized into Caco-2 cells

Optical slicing in the z-axis permitted localization of vesicles inside cells at different levels. Figure 1 shows the distribution of Texas Red–labeled Fe-Tf (Figure 1A) and apo-Tf (Figure 1B) relative to the green ToPro-1–stained nuclei. The images have been reconstructed to allow examination from a lateral perspective. The distribution of vesicles containing apo-Tf is different from those containing Fe-Tf: apo-Tf appears in a perinuclear region and Fe-Tf in a more basal region. A similar pattern was seen when cells were

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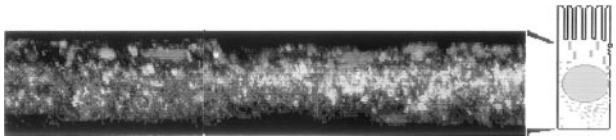

Figure 1. Distribution of apo-Tf–Texas Red and Fe-Tf–Texas Red in Caco-2 cells. Caco-2 cells grown in monolayers were incubated in the basal chamber with either 0.1 μm apo-Tf–Texas Red or 0.1 μm Fe-Tf–Texas Red for 20 minutes. The cell layers were washed and processed, and the nuclei were stained with ToPro-1 and examined by confocal microscopy as described in materials and methods. Figure 1A represents the incubation with Fe-Tf and Figure 1B the incubation with apo-Tf. In both panels, the images have been reconstructed to allow examination from a lateral perspective. The lateral view (xz) is made up by the addition of consecutive pixels in the y-axis (total of 1 μm). The nuclei are stained green. The cartoon next to each image depicts the approximate height of the cells in the reconstruction.

incubated simultaneously with apo-Tf–Texas Red and Fe-Tf–Bodipy (data not shown).

It is possible that apo-Tf or Fe-Tf could initiate an endocytosis pathway that would dictate the path for subsequent Tf binding to the basal surface. Also, Fe-Tf after endocytosis and delivery of its Fe will become apo-Tf and could then travel to the perinuclear region. To consider these possibilities, we examined the distribution of apo-Tf–Texas Red and Fe-Tf–Bodipy by confocal microscopy after incubation for 7, 14, and 21 minutes (Figure 2A and C). After 21 minutes, the basal chamber contents were removed and the other form of Tf was added (Figure 2B and D). In these experiments, for each of the panels the fluorescence was normalized to the greatest intensity seen at any 1 of the 3 time points. Again, apo-Tf accumulated in a perinuclear region of the cell (Figure 2A), and Fe-Tf accumulated in a basal compartment (Figure 2C). After 21 minutes, the original Tf was chased from the cells and the newly added Tf went to the expected location: apo-Tf to the perinuclear area (Figure 2D) and Fe-Tf to the basal region (Figure 2B). In neither instance did changing the basal chamber contents to the other Tf affect the location of the labeled Tf already within the cell.

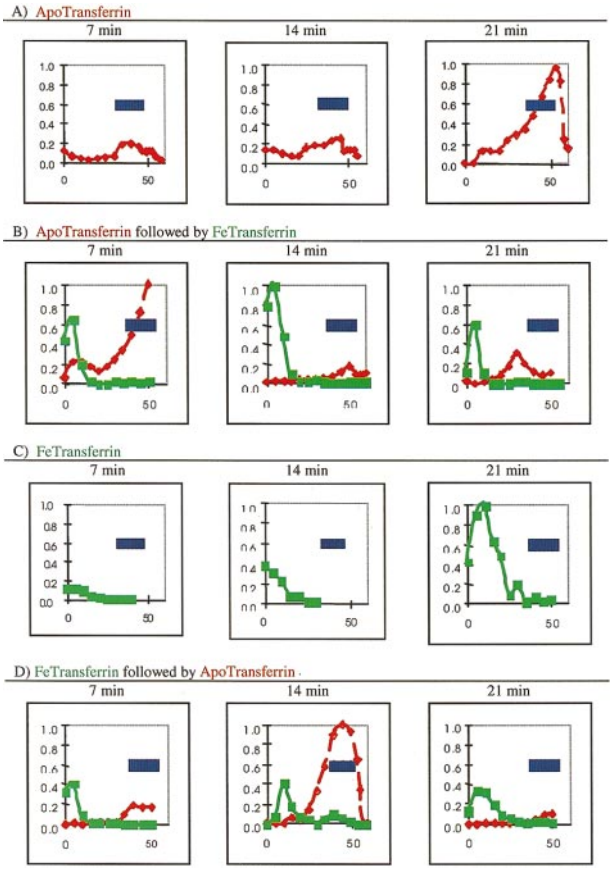

Figure 2. The kinetics of the distribution of apo-Tf–Texas Red and Fe-Tf–Bodipy in Caco-2 cells. Multiple Caco-2 cell monolayers were incubated either with 0.1 μm apo-Tf–Texas Red (panels A and B) or 0.1 μm Fe-Tf–Bodipy (panels C and D) and were examined by confocal microscopy after 7, 14, and 21 minutes of incubation (panels A and C). After 21 minutes, the basal chamber contents were changed so that the cells containing apo-Tf–Texas Red were now offered Fe-Tf–Bodipy (panel B) in the basal chamber, and the cells that had seen Fe-Tf–Bodipy were now offered apo-Tf–Texas Red (panel D). The cells were again examined after an additional 7, 14, and 21 minutes (panels B and D). At the indicated times, the cells were then washed and processed, and the nuclei were stained blue with ToPro-3. The graphs on the y-axis show the average intensity of the fluorescent transferrins obtained by image analysis of 2 to 4 independent experiments for each treatment with duplicate monolayers. The fluorescence intensity for each of the 4 panels was normalized to the highest intensity occurring at the 3 times. The x-axis is the distance in arbitrary units from the membrane on which the cells were grown. In each monolayer, 10 to 23 optical slices were analyzed. The cell monolayers averaged 251 cells per monolayer. The blue bar indicates the area encompassed by the nuclei.

Discussion

There has been controversy regarding the role of Tf in mediating iron transport across the intestine. In Caco-2 cells, both apo-Tf and Fe-Tf stimulate release of iron from the basal surface.[5,6,8,9,11] In Caco-2 cells, apo-Tf will bind to the transferrin receptor, compete with the binding of Fe-Tf, and has a transit time, twice that of Fe-Tf.[10] The present studies demonstrate that Caco-2 cells internalize apo-Tf and Fe-Tf and transport each to a different compartment. These results suggest that the intracellular route of Tf is determined early in the interaction of the Tf with the cell. Presumably, the endocytosis of apo-Tf to a more apical position in the cell explains the longer dwell-time of the apo-Tf in the cell than the Fe-Tf.

The iron transporter, Nramp2, is localized in the brush border of intestinal epithelium.[14] Newly absorbed iron has been demonstrated in the apical portion of intestinal epithelium. The endocytosis of apo-Tf toward the apical portion of the cell could position apo-Tf to interact with newly absorbed iron. Caco-2 cells exhibit transcytosis of basal surface–derived Tf-containing endosomes, allowing for exchange between the apical and basal surfaces.[15] Isoelectric focusing of Caco-2 cells shows newly absorbed ^{59}Fe on apo-Tf offered to the cells.[6] A model of iron transport could involve the endocytosis of apo-Tf to a compartment where the apo-Tf could acquire iron. The now Fe-Tf would then undergo exocytosis, releasing Fe at the basolateral surface. The constitutive transport of iron could result from transcytosis of apical vesicles, a process that would be stimulated by the presence of apo-Tf.

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