Identification of Cell Adhesion Molecules in the Human Follicle-Associated Epithelium That Improve Nanoparticle Uptake into the Peyer’s Patches

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ABSTRACT

The aim of this study was to identify cell adhesion molecules that could serve as targets of the human follicle-associated epithelium (FAE) overlying Peyer’s patches and to assess nanoparticle uptake levels across this epithelium. We first studied the expression of the mouse M-cell marker β1-integrin and used a model of human FAE derived from intestinal epithelial Caco-2 cells and Raji B-cells to identify additional potential targets by cDNA array. The protein expression of potential targets in the model FAE and in human ileal FAE tissues was quantified by immunofluorescence. Integrin targeting was studied by investigating the transport of Arg-Gly-Asp (RGD)-coated (integrin-binding), Arg-Gly-Glu (RGE)-coated (nonintegrin-binding), and uncoated nanoparticles across ileal specimens mounted in Ussing chambers. Both β1-integrin and the cell adhesion molecule CD9 were more abundantly expressed in the model and human FAE compared with the Caco-2 control cells or villus epithelium (VE). Uncoated nanoparticles were not taken up across either FAE or VE. General integrin targeting with RGD improved the nanoparticle transport dramatically across the FAE and to a lower extent across the VE. Compared with RGE, RGD improved transport 4-fold across the FAE. There was no difference in the transport of RGD- and RGE-coated nanoparticles across the VE. In conclusion, β1-integrin and CD9 were identified as targets in human FAE. The difference in RGD- and RGE-mediated transport across the FAE, but not the VE, suggests that a specific integrin interaction was the dominating mechanism for improved nanoparticle uptake across the FAE., whereas charge interaction contributed substantially to the improved VE uptake.

Oral delivery of whole antigens is by far the most effective method of inducing gastrointestinal mucosal immune responses (O’Hagan and Rappuoli, 2006). There are promising data from animal studies using microparticulate carriers for delivery also of subunit antigens, but so far, few of these have succeeded in inducing effective immune responses in humans (Mutwiri et al., 2005). Therefore, it is being increasingly questioned whether encapsulated antigens can be taken up in sufficient quantities to induce adequate immune responses. Studies of commonly used biogradable particles (polylactic-coglycolic acid) have shown that less than 0.01% of the given dose is taken up into human cell cultures or animal tissues (Brayden, 2001). These data suggest that an improvement in the efficacy of particle uptake is required before subunit oral vaccination becomes a feasible option. In this perspective, surprisingly little is known of particle uptake in the human intestine.

Targeting to specific surface receptors on epithelial cells by use of receptor ligands on the particle surface has been successful in improving uptake of proteins across intestinal epithelial cells in culture (Russell-Jones, 2004). However, in vivo, the mucus layer most probably limits particle uptake across the human villus epithelium (VE). In contrast, antigen uptake across the follicle-associated epithelium (FAE), covering Peyer’s patches (PP), is facilitated by the absence of mucus-producing Goblet cells and a reduced expression of hydrolases. A prominent feature of this epithelium is also the presence of specialized M-cells (Owen, 1999) that transport...
intact protein antigens, as well as particles, viruses, and bacteria across the epithelial cell layer (Neutra, 1999; Owen, 1999). We have recently shown an increased transport of antigens and bacteria across the human FAE compared with the VE (Keita et al., 2006).

Thus, the FAE and M-cells are prime targets when aiming at improved particle uptake and immune responses. Targeting of particles to mouse M-cells by use of various ligands, including Ulex Europaeus agglutinin 1 (Foster et al., 1998) and immunoglobulins (Smith et al., 1995), increased adherence, and transcytosis of particles across M-cells. However, the magnitude of these effects has not been assessed, and the size of the particulate carrier and accessibility of the receptor seem critical for the outcome (Mantis et al., 2000; van der Lubben et al., 2001).

In humans, little is known about surface proteins that could act as specific targets in human FAE. Lectins such as the sambucus nigra antigen seem to predominantly stain polysaccharides in the human FAE (Jepson et al., 1996), but the expression of these sugar moieties varies considerably along the intestine, as well as among individuals (Gebert et al., 1996; Sharma et al., 1996). Two putative human M-cell markers have been reported, cathepsin E (Finzi et al., 1993) and the sialyl Lewis A antigen (Giannasca et al., 1999), but unfortunately, these results could not be reproduced (Wong and Florence, 1998). Surprisingly, the expression of these data were inconclusive regarding site of uptake (Husain and Florence, 1998). Interestingly, the expression of β1-integrin has not yet been reported in human FAE, and the effect of targeting particles to β1-integrin has not been investigated in human or mouse intestine.

The aim of this study was to identify cell adhesion molecules that could serve as targets in the human FAE. We first selected the proteins expressed in the model FAE cell monolayers (Schulte et al., 2000; des Rieux et al., 2005). Studies in rats have suggested that targeting of particles to β1-integrin may improve uptake across the intestinal epithelium, although these data were inconclusive regarding site of uptake (Husain and Florence, 1998). Surprisingly, the expression of β1-integrin has not yet been reported in human FAE, and the effect of targeting particles to β1-integrin has not been investigated in human or mouse intestine.

The study was approved by the local committee of human ethics in Linköping, and all subjects gave their informed consent. Specimens were obtained from the ileum next to the ileocaecal valve during ileocolonic surgery of eight patients diagnosed with colon cancer at the University Hospital of Linköping. The median age of the patients (five men and three women) was 67 years (range, 35–87). Immediately following dissection, the specimens were snap-frozen in liquid nitrogen and stored in Cryomount. Cryosections (5 μm) were cut using a microtome (Leica, Wetzlar, Germany) and dried at room temperature on glass slides overnight, before storage at −70°C. Sections were prepared for immunofluorescence by fixing in ice-cold acetone for 10 min, followed by drying at room temperature.

Immunostaining of Fixed Cell Cultures and Cryosections. All cryosections and formaldehyde-fixed cell cultures were micro-waved for 10 min in 10 mM citrate, pH 6 (β1-integrin), or 1 mM EDTA, pH 8 (CD9), as recommended by the manufacturers of the antibodies. After cooling to room temperature, the sections/cells were rinsed several times with distilled water, washed in HBSS, and permeated with 0.2% Triton X-100 for 5 min (β1-integrin) or with Cryofix (Merck, Darmstadt, Germany) for 2 min (CD9). After blocking with HBSS containing 1% fetal calf serum, the sections/cells were

Materials and Methods

Cell Culture. Caco-2 cells (ECACC, Salisbury, UK), clone 1 Caco-2 cells (Dr. Maria Rescigno, University of Milano-Bicocca, Milano, Italy), and the human Burkitt’s lymphoma cell line Raji (American Type Culture Collection, Manassas, VA) were maintained as described previously (Gullberg et al., 2000). For both RNA isolation and transport experiments, Caco-2 cells were grown on polycarbonate filters (pore size, 3.0 μm; Costar, Bædevæn, The Netherlands), which were coated with Matrigel (BD Biosciences, San Jose, CA). To obtain the model FAE, 5 × 10^6 Raji cells were added to the basolateral chamber of the Caco-2 cell monolayers at day 14. The cocultures were maintained for 4 to 5 days (Gullberg et al., 2000). Monocultures of Caco-2 cells grown on filters for 18 to 19 days were used as controls. The transport of fluorescence-labeled polystyrene particles (1 μm; Invitrogen, Carlsbad, CA) was used to verify the function of each batch of model FAE. Transepithelial electrical resistance (TEER) was used for assessment of cell monolayer integrity (see transport experiments below). The cell monolayers were prepared for immunofluorescence studies by 10-min fixation in 4% buffered formaldehyde and subsequent rinsing with Hanks’ balanced salt solution (HBSS with calcium, magnesium, and sodium bicarbonate; Invitrogen). They were stored in HBSS until use.

RNA Isolation, Array Hybridization, and Gene Expression Analysis. For RNA isolation, the cell monolayers were rinsed several times with ice-cold phosphate-buffered saline (PBS; 0.01 M NaPO4; 0.14 M NaCl, 0.003 M KCl) and scraped off the filters into RNase-inhibiting denaturing Solution (Ambion, Austin, TX). Total RNA was isolated with Ambion’s Totally RNA kit according to the manufacturer’s instructions. All RNA samples were DNase-treated, and control PCR was run on all RNA samples to confirm that they were free from genomic DNA. RNA samples from three pooled model FAE and Caco-2 cell batches (six filters in total) were reversibly transcribed to [32P]cDNA and hybridized to cell-adhesion/cell-interaction arrays (BD Biosciences Clontech, Palo Alto, CA), according to the manufacturer’s instructions. The primer mix used for reverse transcription only amplified transcripts from genes present on the array.

Differences in gene expression were analyzed by Atlas Image (BD Biosciences Clontech). Signals lower than the average background signal were filtered out. To compare two or more arrays, the signal intensities of all genes on the arrays were normalized according to the manufacturer’s instructions.

Isolation of Peyer’s Patches from Human Ileum and Preparation of Cryosections. The study was approved by the local committee of human ethics in Linköping, and all subjects gave their informed consent. Specimens were obtained from the ileum next to the ileocaecal valve during ileocolonic surgery of eight patients diagnosed with colon cancer at the University Hospital of Linköping. The median age of the patients (five men and three women) was 67 years (range, 35–87). Immediately following dissection, the specimens were placed in ice-cold oxygenated Krebs-Ringer buffer (115 mM NaCl, 25 mM NaHCO3, 2 mM K2HPO4, 1.25 mM CaCl2, 1.2 mM MgCl2), pH 7.4, and taken to the laboratory for the experiments. A pathologist examined the specimens, which all showed macroscopically normal histology. While still immersed in ice-cold buffer, the external muscle and myenteric plexus were stripped from the surgical specimens. After identification in a dissection microscope, transilluminated from below (Keita et al., 2006), regions of VE and FAE were cut out, oriented to give cross-sections of the intestinal wall, and dried at room temperature on glass slides overnight, before storage at −70°C. Sections were prepared for immunofluorescence by fixing in ice-cold acetone for 10 min, followed by drying at room temperature.
stained with primary antibodies to β1-integrin (clone J10, 400 mg/ml; Biogenex, San Ramon, CA) or CD9 (clone 72F6, 1:100; Serotec, Oxford, UK). The primary antibodies were detected with secondary goat-anti-mouse IgG1 antibodies conjugated to Alexa 488 (Invitrogen). Antibodies to CD20 (clone L26, 1:100; Biomena, Foster City, CA) were used to visualize CD20-positive B-cells in lymphoid follicles and M-cells (Yamanaka et al., 2001). CD20 was detected using Alexa568 goat-anti-mouse IgG2a (Invitrogen). Sections incubated with isotype-matched antibodies were used as controls.

Immunofluorescence Analysis of Cell Cultures and Human Tissue. The immunofluorescence of β1-integrin and CD9 in cell cultures was analyzed by optical sectioning in a confocal laser scanning microscope (Leica), using the same settings for the model FAE and Caco-2 epithelium. Fluorescence intensity profiles were made in ImageJ software (http://rsb.info.nih.gov/ij). The human tissue sections were analyzed using an Axioplan fluorescence microscope (Carl Zeiss GmbH, Jena, Germany). Pictures were captured with a CCD camera (Hammamat C4542–95B; Hammamat Corporation, Bridgewater, NJ), using the same gain and exposure times for all sections on the same slide. Relative fluorescence intensities of the epithelium were measured using ImageJ software. Two slides were prepared on different occasions for each protein and each patient. Four to eight sections, representing two to three different cell layers, were analyzed per patient. In each tissue section, all types of epithelia, FAE, VE, and villus epithelium adjacent to dome (VED) were represented, and their fluorescence intensities relative to each other was determined within the same section. Ten measurements of cell membrane fluorescence intensities were performed on equal areas of the different type of epithelia in each section. Background values obtained from control sections on each slide were subtracted from the true measured fluorescence intensities found on the same slide. The data were normalized to compare protein expression among the patients. The fluorescence intensity for each sample from a particular patient was divided by the sum of the fluorescence intensities of the total tissue sections for that patient. By two-way ANOVA, we analyzed the difference in fluorescence intensities between each type of epithelia from the same patient, and in each type of epithelia between different patients. The tissue obtained from two patients lacked villus epithelium. For these patients, only the fluorescence intensities of FAE and VED are reported.

Preparation of RGD- and RGE-Coated Polystyrene Nanoparticles. Yellow-green fluorescent polystyrene particles (average diameter, 200 nm) were purchased from Polysciences Inc. (Warrington, PA). Pluronic F108 modified with a thiol-specific pyridyl disulfide group (Cell Link) was kindly donated by Allvio Inc. (Lake Forest, CA). Two polypeptides containing the RGD motif (Cys-Gly-Arg-Gly-Asp-Ser-Tyr) and the RGE motif (Cys-Gly-Arg-Gly-Glu-Ser-Tyr) were synthesized at the Department of Medicinal Chemistry, Uppsala University. The Pluronic surfactant was adsorbed onto the particles by a hydrophobic interaction, and the peptide was subsequently adsorbed onto the particles of the surfactant adsorbed. After 2 h, the serosal buffer was withdrawn, and the number of particles was measured by fluorescence-activated cell scan. Analysis of transport data were performed using one-way ANOVA and Student’s t tests. For qualitative assessment of particle uptake, the tissues were washed with PBS (composition, see above) to remove excess particles after 15 and 45 min and the specimens were fixed with 4% formaldehyde overnight. Subsequently, the tissues were rinsed with PBS, labeled with 22 nM phalloidin-Alexa Fluor 594 (Invitrogen) for 30 min, washed repeatedly with PBS, and the whole tissue was visualized in PBS-containing chambers under a Nikon Eclipse E600W confocal laser scanning microscope (Nikon, Melville, NY).

Results

Increased Expression of β1-Integrin in Human FAE. Immunofluorescent staining of β1-integrin in human Peyer’s patches revealed increased expression of β1-integrin in human FAE compared with the surrounding villi. In the FAE, β1-integrin was strongly expressed all around the basolateral face of the epithelial cells (Fig. 1, A and B). β1-Integrin expression could also be found on the apical surface of the FAE, typically where CD20-positive cells infiltrated the epithelium (Fig. 1, D and F) but occasionally also in epithelial cells not associated with CD20-positive lymphocytes (Fig. 1E). In contrast, β1-integrin predominantly lined the basal part of the epithelial cells of the surrounding villi and was strongly expressed on cells in the lamina propria. This expression pattern compares well with previous studies on β1-integrin expression in human intestinal villi (Lussier et al., 2000). Thus, it seemed that β1-integrin had an increased accessibility in human FAE and was indeed a promising target. The next step was to identify additional targets by cDNA array using our previously established model FAE.

Increased Gene Expression of Adhesion and Actin-Associated Proteins in the Model FAE. There were differences in the expression of 18 (7%) of the 256 genes represented on the cell adhesion/cell interaction array between the model FAE and the Caco-2 epithelium. These genes code for proteins involved in cell adhesion, epithelial cell polarity and differentiation, actin dynamics, and extracellular matrix re-

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modeling (Fig. 2). In particular, the expression of cell adhesion molecules CD9, CD4, \( \alpha_5 \)-integrin, and epithelial differentiation markers plakoglobin, desmoplakin II/III, and Wnt-5a was induced in the model FAE. The expression of E-cadherin was down-regulated. Among the genes coding for proteins involved in extracellular matrix remodeling, two matrix metalloproteinases, MMP14 and MMP17, were induced. In agreement with a previous study, we did not find an up-regulation of \( \beta_1 \)-integrin transcripts in the model FAE. Hamzaoui et al. (2004) nicely demonstrated that an increased expression of \( \beta_1 \)-integrin in the apical membrane of FAE-like cell monolayers was due to increased maturation of existing precursors, rather than an increase in production of the protein per se.

Assessing the protein expression of all of the genes identified by the array was beyond the scope of this study. The cell adhesion molecules CD9 and CD4 were chosen for further investigation since they exhibited the most pronounced up-regulation in expression and since both of these proteins have been reported to act as coreceptors for viruses and bacteria. CD9 is a tetraspan protein that interacts with integrins in the cell membrane (Berditchevski et al., 1999), and CD4 is a well known coreceptor for human immunodeficiency virus (Dalgleish et al., 1984). Therefore, we compared the expression of these two proteins with the expression of \( \beta_1 \)-integrin in the model FAE.

**Enhanced Protein Expression of CD9 in the Model FAE Compared with the Caco-2 Epithelium.** The protein expression of CD9 was clearly enhanced in the model FAE compared with the Caco-2 epithelium (Fig. 3, A and B). In both epithelia, CD9 was expressed laterally; however, expression in the basal and apical cell membranes was most pronounced in the model FAE. We were unable to find protein expression of CD4 in any of the two epithelia (Fig. 3, C and D). Reverse transcriptase-PCR studies revealed that there were no difference in the number of CD4 transcripts between the model FAE and Caco-2 control epithelium (data not shown). Thus, the increase in CD4 gene expression was falsely detected by the cDNA array. In contrast, the increase in CD9 expression was confirmed by reverse transcriptase-PCR, with a ratio of 1.5 CD9 mRNA transcripts in the model FAE compared with the Caco-2 epithelium.

The expression of \( \beta_1 \)-integrin was evenly distributed in the Caco-2 cells, but in the model FAE, it was highly expressed in the apical membrane (Fig. 3, E and F), as previously reported (des Rieux et al., 2005). Next, we analyzed the expression of CD9 in human Peyer’s patches and assessed the expression levels of both CD9 and \( \beta_1 \)-integrin in different individuals.

**The Expression of Both \( \beta_1 \)-Integrin and CD9 Is Higher in Human FAE Than in Villus Epithelium.** Qual-
 iterative assessment of CD9 expression in human Peyer’s patches revealed that the FAE was indeed positive for CD9, whereas the VE was negative (Fig. 4A). In addition, CD9 was expressed by cells in the lamina propria in all specimens and could also be found in the lymphoid follicles. The expression of CD9 in VE is in agreement with a previous study of CD9 expression in the human small intestine (Sincock et al., 1997). In concordance with the results obtained in cell cultures, CD9 expression was found in both the apical and basolateral membranes of the epithelial cells in the FAE (Fig. 4B).

Semiquantitative assessment of the expression levels of β1-integrin and CD9 in the eight individuals studied confirmed our qualitative findings (Fig. 5). The expression levels of both proteins were 2- to 3-fold higher in the FAE than in the VE. In several of the specimens obtained, the villi adjacent to the dome (Fig. 5A, VED) also expressed CD9, whereas villi situated further away from the dome (VE) displayed no or low expression (Fig. 5B). In contrast, there was no difference in β1-integrin expression between the VED and VE (Fig. 5C).

Increased Transport of Integrin-Adherent Particles across the Model and Human FAE. Our expression data suggested that our initial proposal to target β1-integrin would indeed be a feasible strategy to improve uptake into human Peyer’s patches. In addition to β1-integrin, we identified an up-regulation of the α5- and α6-integrin subunits on the mRNA level in the model FAE (Fig. 2). Therefore, we chose a general integrin-targeting sequence to maximize adherence and the uptake and transport of nanoparticles. The RGD peptide motif (Arg-Gly-Asp) is known to compete with Yersinia invasin A in binding to β1-integrin (Leong et al., 1995) but also to recognize many other integrins, including the α5- and α6-integrin subunits (Ruoslalhi, 1996). CD9 also seemed promising, but to date, no ligands have been reported for human CD9. It was judged beyond the scope of this study to identify such molecules; thus, we decided to study the effects of targeting integrins.

Optical sectioning of whole-tissue specimens in a confocal microscope showed uptake of RGD-coated particles across the FAE as early as 15 min after application (Fig. 6A). After 45 min, the RGD-coated particles had crossed the FAE and were found deep inside the dome (Fig. 6B), whereas particles
null
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Yersinia was responsible for the adherence and uptake of bacteria across the mouse FAE (Clark et al., 1998). Recently, a previous study also identified an mRNA transcript of a tetraspan protein (TM4SF3) with specific expression in the human FAE (Lo et al., 2004). Thus, CD9 and possibly other tetraspan proteins may play a role in the regulation of integrin-mediated uptake.

Regardless of the tetraspan interaction, the relationship between integrin expression levels and functionality was evident in this study. The median difference and variability in RGD-mediated nanoparticle transport between the FAE and the VE (Fig. 8) compares well with the mean difference and variability in \( \beta_1 \)-integrin expression between the FAE and the VE (Fig. 5). Our transport data also clearly demonstrate the functional involvement of integrins in the particle uptake. There was a 4-fold increase in transport between RGD- and RGE-coated particles across the FAE, which had an increased expression of \( \beta_1 \)-integrins. However, no significant difference was found between RGD- and RGE-coated particles in VE, where we found no \( \beta_1 \)-integrin expression. The involvement of other coexpressed RGD-binding integrins in the uptake cannot be excluded. Their expression in human FAE is not known.

Because RGD and RGE induced nanoparticle transport across the VE to the same extent, it was conceivable that a mechanism other than integrin interaction was of importance for this uptake. Because hydrophobic latex particles coated with only the hydrophilic Pluronic linker were not taken up at all (Fig. 6), we could exclude the involvement of an unspecific interaction with the latex particle surface. Furthermore, we could not find any difference in particle size or aggregation tendency between Pluronic-coated and peptide-Pluronic-coated particles during our initial characterization studies (Andersson et al., 2005), which excluded a size effect. Thus, the most likely explanation is a charge interaction between the peptide-coated particles and the VE cells or mucus. Both RGD and RGE contain amino acids that confer both positive (arginine) and negative (aspartic and glutamic acid) charge at pH 6 to 7.5, whereas the Pluronic linker is unchanged in this pH range. In addition, free RGD peptide inhibited both RGD- and RGE-mediated uptake. In the FAE, however, the transport of RGD-coated particles was much higher than the transport of RGE-coated particles. Judging from these data, the contribution of charge to the improved transport across the FAE was less than 25%, indicating that the specific interaction with integrins was of major importance (Fig. 8).

Our data certainly points to the necessity for a specific interaction when aiming at improved uptake into Peyer’s patches. Despite the improvement in nanoparticle transport seen with both RGD and RGE, we did not find a significant difference in the uptake of RGE-coated particles across the FAE compared with the VE. This further supports that the FAE mediated an increased particle uptake by the specific expression of integrins and not by unspecific mechanisms, such as an increased accessibility or a higher transport capacity. The difference in CD9 expression levels between FAE and VE were even more prominent than for \( \beta_1 \)-integrin, and it would be exciting to investigate the selectivity of a CD9 targeting sequence for the human FAE, once a ligand has been discovered.

A possible application of our findings would be to improve the uptake of subunit oral vaccine formulations. The crucial question is, therefore, if the uptake of particles, even after use integrin targeting to enhance nanoparticle uptake into human Peyer’s patches. To our knowledge, our study is the first ever that provides data on targeting to the human FAE.

It is known that various integrins mediate, and potentially regulate, uptake of particulate antigens in cell culture and animal studies (Kerr, 1999; Schulte et al., 2000). An earlier study showed that \( \beta_1 \)-integrin expressed apically on M-cells was responsible for the adherence and uptake of Yersinia bacteria across the mouse FAE (Clark et al., 1998). Recently, Tyrer et al. (2006) confirmed and extended this finding by showing that \( \beta_1 \)-integrin associates with \( \alpha_9 \)-integrin in mediating integrin-dependent uptake of bacteria across mouse M-cells. In our study, \( \beta_1 \)-integrin stained the apical membranes of dome epithelial cells associated with CD20-positive lymphocytes. Thus, the transport of RGD-coated particles across the FAE was possibly mediated by M-cells. However, apical expression of \( \beta_1 \)-integrin was occasionally also found in dome epithelial cells not associated with CD20-positive lymphocytes, and it cannot be excluded that integrins expressed on other FAE cells contributed to the increased uptake. The presence of integrins in human FAE has not been reported previously, but it is known that their expression is tightly regulated (Lussier et al., 2000).

The tetraspan proteins, such as CD9, are known to modulate both the location and the conformation of many proteins, including integrins, through binding and interactions within so-called tetraspanin webs in the cell membrane (Berditchevski and Odintsova, 1999). Specifically, earlier studies have demonstrated an association between CD9 and \( \beta_1 \)-integrin precursors in epithelial cells (Rubinstein et al., 1997), suggesting that CD9 is involved in maturation and delivery of \( \beta_1 \)-integrins to the cell membrane. CD9 has also been shown to play a role in protein kinase C-mediated integrin signaling (Zhang et al., 2001) and focal adhesion kinase-induced reorganization of the cortical actin cytoskeleton (Berditchevski and Odintsova, 1999). Both of these events are critical for integrin-mediated uptake of particulate antigens (Dramsi and Cossart, 1998). Interestingly, a previous study also identified an mRNA transcript of a tetraspan protein (TM4SF3) with specific expression in the human FAE (Lo et al., 2004). Thus, CD9 and possibly other tetraspan proteins may play a role in the regulation of integrin-mediated uptake.

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targeting, will be sufficient to initiate an immune response. Two hours after administration of RGD-coated particles, 1% of them had crossed the entire FAE and underlying tissue (Fig. 8). We are not aware of any previous studies of intestinal particle transport across human tissues using the Ussing chamber technique; thus, comparisons are difficult. However, our data can possibly be qualitatively compared with animal studies where particles have been instilled in ligated intestinal loops. From our confocal microscopy studies, we approximate that at least 50 RGD-coated particles of sizes up to 5 µm were found in each dome covered by FAE after 45 min. During experiments with ligated loops in the mouse intestine, using cross-linked starch particles, only one to three particles (2 µm in size) were taken up in 4 of the 10 studied domes after 45 min (Stertman, 2004). Still, a lower dose of starch particles than the one used in the loop has been shown to initiate a prominent immune response after oral administration (Strindelius et al., 2002). The RGD-mediated uptake of 50 or more particles in our study was at least 20 times higher, but whether it will be sufficient to initiate an immune response after oral administration remains to be seen.

In conclusion, by using our recently introduced technique to obtain human FAE (Keita et al., 2006), we have been able to investigate the transport of nanoparticles into human Peyer’s patches and assess the relative contribution of FAE and VE to nanoparticle uptake in the human intestine. We show that uptake of nanoparticles is a rare event, also in human intestine, but that the uptake into Peyer’s patches may be dramatically improved by introducing an integrin targeting moiety on the particles. Apart from integrins, the integrin-associated cell adhesion molecule CD9 has an increased expression in human FAE and also holds as a potential target, once a targeting ligand has been identified. These findings provide a platform for improving the intestinal uptake and effects of oral vaccine formulations in humans.

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References


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