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Editorial

Roadblock for antigens – take a detour via M cells

See Related Article: Markov, A.G., Falchuk, E.L., Kruglova, N.M., Radloff, J., Amasheh, S. Claudin expression in follicle-associated epithelium of rat Peyer's patches defines a major restriction of the paracellular pathway. *Acta Physiol* 215, 112–119.

In this issue of *Acta Physiologica*, Markov *et al.* describe the electrophysiological properties, the permeability for macromolecules, the biochemical composition and the morphological arrangement of major tight junctional proteins of the follicle-associated epithelium covering the Peyers's patches in rat small intestine. In comparison with 'normal' villous epithelium from regions outside the Peyer's patches, the follicle-associated epithelium displayed a higher transepithelial electrical resistance. This was particularly seen when the differences in architecture of the follicle-associated and the villous epithelium were taken into account as the measured resistance [R in Ω] of a tissue sample is inversely related to its area. After correction for area, the tissue resistance [R_{tissue} in $\Omega \cdot cm^2$] was nearly fourfold higher in the mucosal layer above the Peyer's patches. Sophisticated one-path impedance spectroscopical analysis, which is based on a model, in which the ohmic resistance of the epithelium is connected in parallel with a capacitance (so that its resistance becomes frequency-dependent), whereas the ohmic resistance of the subepithelial layer(s) is independent from the frequency of the applied current pulses, revealed that both a higher epithelial resistance and a higher subepithelial resistance contributed to the increased electrical 'tightness' for ionic currents. This was paralleled by a reduced paracellular flux of different macromolecular markers. Western blots and confocal microscopical analysis revealed an increased expression of 'sealing' tight junctional proteins such as claudin-1, claudin-4, claudin-5 and claudin-8. Consequently, there is a strong barrier in the follicle-associated epithelium opposing the paracellular flux of macromolecules. This forces the transcellular uptake of antigens via transcytosis by the M cells covering the surface of the Peyer's patches.

The Peyer's patches are part of the gut-associated lymphoid tissue (GALT), which is considered as the largest immune organ of mammals (Pabst & Rothkötter 2006). They are covered with so-called M cells, epithelial cells with poorly developed apical microvilli (Kraehenbuhl & Neutra 2000), which are known to deliver antigens or macromolecular pathogens such as prions (Kujala *et al.* 2011) to lymphocytes within the lymph follicles of the Peyer's patches via transcytosis. Differences in the basal electrophysiological properties of the follicle-associated epithelium have already been described more than 20 years ago (Brayden & Baird 1994), when it was found in rabbit small intestine that this epithelium – in comparison with villous epithelium –exhibits a fivefold higher tissue resistance and a reduced basal short-circuit current (i.e. a low basal electrogenic transport rate) when mounted in Ussing chambers. Furthermore, the electrical response to the stable acetylcholine derivative carbachol, a Ca^{2+}-dependent secretagogue, was reversed into a fall in short-circuit current and neither stimulation of the cAMP- nor the cGMP-signalling pathway was able to induce an anion secretion in this region of the intestinal mucosa (Brayden & Baird 1994). However, the reasons for these differences remained unknown.

The study presented here by Markov *et al.* now gives an explanation at least for the distinct properties of the epithelial barrier based on differences in the architecture of the tight junctions. These cellular contacts surround in a belt-like fashion the epithelial cells at their apical pole. They seal the intercellular spaces between epithelial cells by forming strands which interact with the strands of neighbouring cells. Tight junctions are composed of different transmembrane proteins such as claudins, occludins and others supported by peripheral scaffolding proteins (like e.g. ZO-1) and are connected to the cytoskeleton (Van Itallie & Anderson 2014). Interestingly, the family of claudins does not only contain barrier-forming members (such as claudin-1) establishing the 'fence' function of the tight junctions, but also contains pore-forming members mediating the paracellular flux of cations (e.g. claudin-2) or anions (e.g. claudin-7), thus contributing to the so-called gate function of these cellular contacts (Günzel & Yu 2013). The situation is even more complicated at sites, where three epithelial cells meet. At these tricellular junctions, the protein tricellulin is located forming a barrier for the paracellular passage of large molecules (Krug *et al.* 2009). All claudins found in the recent study of Markov *et al.* to be preferentially expressed in the follicle-associated epithelium, that is claudin-1, claudin-4, claudin-5 and claudin-8, belong to the functional class of barrier-forming claudins. Consequently, there is not

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only a gradient in the composition of the tight junctions in intestinal epithelia along the longitudinal axis of the gut with increasing expression of the barrier-forming junctional proteins claudin-1, claudin-3, claudin-4, claudin-5 and claudin-8 in the colon compared to the small intestine as revealed by another study of this German–Russian cooperation (Markov *et al.* 2010), but there are also regional differences within the small intestine, especially above the dome region of the Peyer's patches. An interesting question to be addressed will probably be the identification of the regulatory factors [and their cellular source(s)] which determine the expression and assembly of tight junctional proteins with either gate or fence function.

The present experiments give an explanation for the observation that large antigens have to be transported via transcytosis to lymphocytes residing in the lymph follicles of the Peyer's patches in order to induce a functional immune response, whereas smaller antigens might also be taken up by non-specialized epithelial cells (Howe *et al.* 2014). Intestinal absorption of antigens is the prerequisite for the development of food allergy, a disease with increasing incidence, where the balance between mucosal tolerance and mucosal defence mechanisms is disturbed (Brandtzaeg 2010). The enhanced 'tightness' of the intercellular epithelial contacts in the follicle-associated epithelium forces larger antigens to be taken up via transcytosis and restricts fast uptake via paracellular diffusion. The special arrangement of cellular contacts in the follicle-associated epithelium suggests that the delayed delivery of antigens to immune competent cells within the Peyer's patches might play a role for physiological functions of the gut-associated lymphoid tissue. Thus, an interesting research topic will probably be the study of the regulation of the composition and/or the morphology of the tight junctions above subepithelial immune competent cells during food allergy or under inflammatory conditions.

Conflict of interest

I declare no conflict of interests.

M. Diener
Institute for Veterinary Physiology and Biochemistry, Justus Liebig University Giessen, Giessen, Germany
E-mail: martin.diener@vetmed.uni-giessen.de

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