

Endotoxin tolerance in intestinal epithelial cells with

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How is the intestinal mucosa composed?

Mathias W. Hornef: Intestinal epithelial cells (IECs) line the surface of the intestine and thereby represent the physical barrier between the sterile subepithelial tissue and the nutrient-containing and microbially colonized intestinal lumen. Enterocytes facilitate degradation, internalization, and transport of fluid and nutrients to provide the body with energy, water, and essential substrates. Also, they have to endure a highly diverse phys-

iological microflora and are the first to potentially encounter enteropathogenic bacteria. The human enteric microflora has been estimated to encompass more than 400 bacterial species and the number of bacterial cells by far outnumber the amount of total body cells (1). The microflora fulfills important physiological functions such as the production of essential vitamins and protection from enteropathogen infection.

Do intestinal epithelial cells recognize bacteria?

Mathias W. Hornef: The recent discovery of receptors that recognize conserved microbial structures and stimulate immune functions has fueled research in the field of infection-related immune responses. The family of Toll-like receptors (TLRs) is the best defined group of innate immune receptors. Among those TLR4 recognizes bacterial lipopolysaccharide (LPS), essential cell wall constituent of all gram-negative bacteria. We could recently show that not only professional immune cells but also differentiated and polarized intestinal epithelial cells express TLR4 and exhibit remarkable LPS susceptibility (2). Much to our surprise, the localization of the TLR4 molecule in intestinal epithelial cells was restricted to the Golgi apparatus and receptor activation required ligand internalization and intact cell traffic (3). This is in contrast to the situation in macrophages which carry TLR4 on the plasma membrane. We could recently confirm this

observation *in vivo*: isolated primary mouse intestinal epithelial cells similarly expressed TLR4 restricted to an intracellular compartment and readily internalized extracellular LPS (4). However, the question whether primary intestinal epithelial cells respond to LPS was more difficult to answer since isolated primary intestinal epithelial cells cannot be propagated and rapidly lose viability. Given the constant exposure to microbial substances within the intestinal tract, researchers had argued before that intestinal epithelial cells do not express or at least do not respond to LPS. A careful analysis, however, revealed an interesting new detail: Whereas isolated intestinal epithelial cells from adult mice were indeed resistant to LPS stimulation, cells taken from fetal tissue readily responded. Even more striking, cells isolated only hours after birth showed spontaneous cellular activation with production of chemokines.

How can the postnatal loss of of LPS responsiveness be explained?

Mathias W Hornef: Both, the primary transient activation as well as the subsequent loss of responsiveness characterize what had originally been described in macrophages using the term "endotoxin tolerance". Interestingly, tolerance induction with one TLR ligand has been shown to also affect signaling via other TLRs, a phenomenon called cross-tolerance. Tolerance induction might therefore be regarded as some kind of global TLR desensitizing mechanism. We therefore aimed to define the stimulus at work during the postnatal epithelial activation. Caesarean-born neonates failed to exhibit epithelial stimulation pointing towards an exogenous stimulus. Also, measurable amounts of microbial substances such as LPS were found in newborn intestinal tissue. Finally, analysis of mice deficient in TLR4 which exhib-

ited only a very weak postnatal epithelial activation identified exogenous LPS as main stimulus *in vivo*. Since the neonate intestine at this time after birth is still sterile, environmental LPS, derived from the mother's lower intestinal tract or the bacterially colonized birth canal seems to evoke epithelial activation and tolerance induction. A large number of mechanisms have been proposed to inhibit TLR signaling in macrophages (5). One mechanism was also identified in intestinal epithelial cells: proteasomal degradation of the TLR4 signaling molecule interleukin 1 receptor associated kinase (IRAK)-1. Posttranscriptional down-regulation of IRAK-1 was noted in a LPS stimulated naïve cell line as well as in primary cells isolated after birth and IRAK-1 overexpression was able to at least partially restore susceptibility *in vitro*.

Why may those findings be important?

Mathias W Hornef: The contribution of intestinal epithelial cells for innate immune recognition and host defense activation against microbial pathogens is still poorly understood. These questions are particularly difficult given the presence of the microbial flora and therefore the continuous presence of so called pattern recognition molecules,

e.g. LPS. The underlying molecular and cellular mechanisms of epithelial innate recognition and the regulatory processes involved might help to better understand host defense activation during enteropathogen infection and intestinal inflammatory processes seen for example in patients with inflammatory bowel disease (IBD).

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