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A New Transporter for Inflammatory Muropeptides

Abstract

Peptidoglycan (PGN), a glyco-polymer of alternating N-acetylglucosamine and N-acetylmuramic acid, is the main component of prokaryotic cell wall. PGN forms a mesh-like structure that provides rigidity through cross linked stem peptides. It has long been known that small fragments of PGN are highly inflammatory. These small PGN fragments, known as muropeptides, are recognized by a number of cytosolic innate immune receptors such as NOD1, NOD2 and NLRP1. These immune receptors activate NF-κB signaling and/ or inflammasome-mediated production of IL-1β, to elicit robust innate immune responses. However, the mechanisms by which small PGN fragments gain access to these cytoplasmic innate immune sensors are not fully understood, yet.

Previous studies have revealed that members of SLC15, a family of oligonucleotide transporters, transport MDP from endosomal compartment to cytoplasm for NOD2-mediated recognition. However, no transporter for larger muropeptides, such as Trachael Cytoxoin (TCT), have been identified. TCT, a disaccharide tetrapeptide derived from DAP-type PGN, is implicated in the cytopathology caused by *B. pertussis* and *N. gonorrhea* and is thought be an agonist for NOD1. To identify transporters for TCT and potential other NOD1 activating muropeptides, we have used *Drosophila* model system. The *Drosophila* innate immune system robustly responds to TCT through two receptors, PGRP-LC, a transmembrane receptor and PGRP-LE, a cytosolic receptor. However, *Drosophila* SLC15 homologs are not responsible for the transport of TCT into the cytoplasm, for recognition by PGRP-LE.

Instead, we find that SLC46 family transporters are involved in the transport of TCT into the cytosol in *Drosophila* cells. We also found that human and mouse SLC46 homologs have similar activity, and support transport of various muropeptides into the cytosol for recognition by NOD receptors. In particular, SLC46A2 robustly supports NOD1-mediated responses to TCT, in multiple human cell lines. The activity of SLC46A2 to deliver NOD ligands is markedly greater than observed for SLC15A1 or SLC15A2. Murine, but not human, SLC46A3 also exhibits activity to transport muropeptides, while SLC46A1, a major folate transporter in the intestine, does not have any muropeptide transporting activity. SLC46A2 display very limited expression, with high levels in the critical epithelia of the thymus, skin keratinocytes, and the human (but not mouse) lung. Interesting, the human but not the mouse trachea responds to TCT, with cytotoxicity, raising the possibility that this species specific response may be due to different SLC46A2 expression patterns. While we were not able to determine the function of SLC46A2 in cell lines, due to its low and negligible expression in all cell lines tested, a knockout mouse is currently being constructed. The analysis of these knockout animals, especially in tissues expressing SLC46A2 will be highly informative as to the *in vivo* role of SLC462 in the cytosolic response to muropeptides.