Department of Microbiology and Immunology

Division of Infectious Genetics
感染遺伝学分野

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Immune cells express multiple Toll-like receptors (TLRs) which are crucial for recognizing pathogen-derived products from microorganisms and viruses. Recent studies have demonstrated that dysregulation in TLR signaling is a critical factor in the onset of autoimmune and autoinflammatory diseases. Nucleic acid(NA) -sensing TLRs detect not only bacterial and viral NAs, but also host-derived NAs. This raises the possibility of a sophisticated regulatory mechanism that finely tunes NA -sensing TLR expression, subcellular localization, and functional activity to avoid excessive responses to self-derived NAs. This concept suggests that elucidating the regulatory mechanisms of NA-sensing TLRs could advance our understanding of the pathogenesis and process of various TLR-induced diseases. Our research endeavors to uncover the regulatory mechanisms that control TLR-mediated recognition of pathogenic ligands, as well as the identification of endogenous ligands. We finally aim to unravel the complex pathogenic mechanisms underlying histiocytosis and autoimmune diseases such as Systemic Lupus Erythematosus, which are hypothesized to be mediated by aberrant TLR activation.

1. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

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Loss-of-function mutations in the lysosomal nucleoside transporter SLC29A3 cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism by which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in *Slc29a3*$^{-/-}$ mice was shown to depend on Toll-like receptor 7 (TLR7), which senses a combination of nucleosides and oligoribonucleotides (ORNs). TLR7 increased phagocyte numbers by driving the proliferation of Ly6C^{hi} immature monocytes and their maturation into Ly6C^{low} phagocytes in *Slc29a3*$^{-/-}$ mice. Downstream of TLR7, FcRγ and DAP10 were required for monocyte proliferation. Histiocytosis is accompanied by inflammation in SLC29A3 disorders. However, TLR7 in nucleoside-laden splenic macrophages failed to activate inflammatory responses. Enhanced production of pro-inflammatory cytokines was observed only after stimulation with ssRNAs, which would increase lysosomal ORNs. Patient-derived monocytes harboring the G208R *SLC29A3* mutation showed enhanced survival and proliferation in a TLR8 antagonist-sensitive manner. These results demonstrated that TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

2. Toll-like receptor 7 signaling modifies the subset pattern and functions of monocytes in NZBWF1 mice.

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and multiple organ damage. Toll-like receptor 7 (TLR7), an innate immune RNA sensor expressed in monocytes/macrophages, dendritic cells (DCs), and B cells, promotes disease progression. We showed that the lupus nephritis in NZBWF1 mice is ameliorated with anti-mouse TLR7 monoclonal antibody in previous study (Murakami and Fukui, Front. Immunol. 2021). To investigate how TLR7 drives lupus nephritis, we generated *Tlr7*$^{-/-}$ (TLR7 knockout, TLR7-KO) NZBWF1 mice. The phenotype of TLR7-KO NZBWF1 mice is similar to anti-TLR7 antibody treated NZBWF1 mice and more reliable. We compared the immune cells in TLR7-KO NZBWF1 mice and WT by single cell RNA-sequencing, and found that an atypical subset of monocytes expands only in the spleen of WT NZBWF1 mice. We also found that the infiltrating patrolling monocytes in glomeruli expressing specific markers, for example, PD-L2. We are analyzing the function of these monocyte subsets focusing on the cytokine production and the interaction with B cells.

3. Thioredoxin-mediated suppression of NLRP1 inflammasome

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Inflammasome sensors detect pathogen- and danger-associated molecular patterns and promote inflammation and pyroptosis. The nucleotide-binding

oligomerization domain-like receptor (NLR) family pyrin-domain containing protein 1 (NLRP1) was the first inflammasome sensor described, and its hyperactivation is linked to autoinflammatory disease and cancer. However, the mechanism underlying the activation and regulation of NLRP1 has not been clearly elucidated and, therefore, in this study, we sought to further investigate this phenomenon. We identified ubiquitously expressed endogenous thioredoxin (TRX) as a binder of NLRP1 and a suppressor of the NLRP1 inflammasome. The cryo-electron microscopy (cryo-EM) structure of a binary complex of NLRP1 and TRX together with mutagenesis studies showed that TRX in the oxidized form was bound to the nucleotide binding domain (NBD) subdomain of NLRP1. This observation highlights the crucial role of redox-active cysteines of TRX in NLRP1 binding. Further cellular assays revealed that TRX suppressed NLRP1 inflammasome activation and, thus, negatively regulated NLRP1. Our data reveal the well-known TRX system as an intrinsic checkpoint for innate immunity and provide opportunities for future therapeutic intervention with NLRP1 inflammasome activation targeting this system.

Publications

Sakaniwa K #, Fujimura A #, Shibata T #, Shigematsu H, Ekimoto T, Yamamoto M, Ikeguchi M, Miyake K, Ohto U, Shimizu T. (#: equally contributed)
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