Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

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Pathogen sensors, such as Toll-like receptor (TLR), play sentinel roles in detecting pathogenic ligands during infection and induce both innate and acquired immune responses. Meanwhile, excessive TLR responses are strongly associated with fatal diseases such as septic shock and autoimmune diseases. For this reason, immune system must strictly control TLR responses to avoid disruption of homeostasis. However, molecular mechanisms involved in TLR regulation are not fully elucidated. We have previously shown that TLRs are regulated by various TLR associating molecules including MD-2, PRAT4A and Unc93B1. Our goal is to uncover molecular mechanism that is indispensable for appropriate TLR responses using genetically engineered mice.

1. Targeting the nucleic acids-sensing TLRs for therapeutic intervention in autoimmune diseases

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TLR7 senses microbial-derived RNA in endolysosome, but can also erroneously respond to self-derived RNA. In fact, it has been reported that TLR7-dependent signaling promote autoimmune diseases. Thus, TLR7 can be therapeutic target. Although antibodies (Abs) are powerful tools for therapeutic intervention, TLR7 has been excluded from targets for Ab-mediated intervention because of its lack of cell surface expression. Despite this expectation, we found an anti-TLR7 Ab dose-dependently inhibits TLR7 responses in dendritic cells, macrophages and B cells. For this reason, we evaluated the therapeutic effect of anti-TLR7 Ab in *Unc93b1*$^{D34A/D34A}$ mice that cause thrombocytopenia, splenomegaly and chronic active hepatitis due to TLR7 hyper-responsiveness, and found that thrombocytopenia in *Unc93b1*$^{D34A/D34A}$ mice was significantly improved by the treatment with anti-TLR7 mAb. Furthermore, splenomegaly and hepatitis in mice treated with the anti-TLR7 mAb were also significantly remedy compared with control antibody.

On basis of these results, we established anti-human TLR7 Ab for blocking human TLR7 responses in vitro. Moreover, we generated human TLR7 transgenic (huTLR7 Tg) mice. We plan to use HuTLR7 Tg mice to evaluate the effects of anti-human TLR7 Ab *in vivo*.

In addition, TLR8 also recognize mouse TLR7 ligands in human and is involved in exacerbation of Rheumatoid Arthritis. Thus, in case of human disease, the anti-human TLR8 Ab that inhibits human TLR8 responses might work in clinical application. For this reason, we also constructed both anti-human TLR8 Abs and human TLR8 transgenic mice to verify our hypothesis.

2. Microbiome ssRNA as an environmental cue to activate TLR13-dependent tissue-protective programs in CD5L^{hi} hepatic macrophages

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Hepatic macrophages maintain liver homeostasis, but little is known about the signals that activate the hepatoprotective programs within macrophages. Here, we show that toll-like receptor 13 (TLR13), a sensor of bacterial 23S ribosomal RNA (rRNA), senses microbiome RNAs to drive tissue-protective responses in CD5L^{hi} hepatic macrophages. Splenomegaly and hepatomegaly developed in the absence of the endosomal RNase, RNaseT2, via TLR13-dependent macrophage proliferation. Furthermore, TLR13 in hepatic Ly6C^{lo} macrophages activated the transcription factors LXRα and MafB, leading to expression of tissue-clearance molecules, such as CD5L, C1qb, and Axl. Consequently, *Rnaset2*$^{-/-}$ mice developed resistance to acute liver injury caused by challenges with acetaminophen and lipopolysaccharide + D-galactosamine. TLR13 responses in *Rnaset2*$^{-/-}$ mice were impaired by antibiotics, suggesting that TLR13 were activated by microbiome rRNAs, which was detected in the sera and hepatic macrophages. Repeated administration of wild-type mice with the TLR13 ligand, rather than other TLR ligands, selectively increased the number of Kupffer cells, which expressed immunoregulatory and tissue-clearance genes as hepatic macrophages in *Rnaset2*$^{-/-}$ mice did. Our results suggest that microbiome ssRNA serves as an environmental cue for initiating tissue-protective TLR13 responses in hepatic macrophages.

3. Aberrant monocytopoiesis drives granuloma development in sarcoidosis

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In sarcoidosis, granulomas develop in multiple organs including the liver and lungs. Although mTORC1 activation in macrophages drives granuloma development in sarcoidosis by enhancing macrophage proliferation, little is known about the macrophage subsets that proliferate and mature into granuloma macrophages. Here, we show that aberrantly increased monocytopoiesis gives rise to granulomas in a sarcoidosis model, in which Tsc2, a negative regulator of mTORC1, is conditionally deleted in CSF1R-expressing macrophages (Tsc2csf1rΔ mice). In Tsc2csf1rΔ mice, common myeloid progenitors (CMPs), granulocyte-monocyte progenitors (GMPs), common monocyte progenitors/monocyte progenitors (cMoPs/MPs), inducible monocyte progenitors (iMoPs), and Ly6Cint CX3CR1low CD14- immature monocytes (iMOs), but not monocyte-dendritic cell progenitors (MDPs) and common dendritic cell progenitors (CDPs), accumulated and proliferated in the spleen. Consistent with this, monocytes, neutrophils, and neutrophil-like monocytes increased in the spleens of Tsc2csf1rΔ mice, whereas dendritic cells did not. The adoptive transfer of splenic iMOs into wild-type mice gave rise to granulomas in the liver and lungs. In these target organs, iMOs matured into Ly6Chi classi-

cal monocytes/macrophages (cMOs). Giant macrophages (gMAs) also accumulated in the liver and lungs, which were similar to granuloma macrophages in expression of cell surface markers such as MerTK and SLAMF7. Furthermore, the gMA-specific genes were expressed in human macrophages from sarcoidosis skin lesions. These results suggest that mTORC1 drives granuloma development by promoting the proliferation of monocyte/neutrophil progenitors and iMOs predominantly in the spleen, and that proliferating iMOs mature into cMOs and then gMAs to give rise to granuloma after migration into the liver and lungs in sarcoidosis.

Publications

Hiranuma R, Sato R, Yamaguchi K, Nakamizo S, Asano K, Shibata T, Fukui R, Furukawa Y, Kabashima K, Miyake K. Aberrant monocytopoiesis drives granuloma development in sarcoidosis. Int Immunol. dxad054. doi: 10.1093/intimm/dxad054. 2023.

Liu G, Haw TJ, Starkey MR, Philp AM, Pavlidis S, Nalkurthi C, Nair PM, Gomez HM, Hanish I, Hsu AC, Hortle E, Pickles S, Rojas-Quintero J, Estepar RSJ, Marshall JE, Kim RY, Collison AM, Mattes J, Idrees S, Faiz A, Hansbro NG, Fukui R, Murakami Y, Cheng HS, Tan NS, Chotirmall SH, Horvat JC, Foster PS, Oliver BG, Polverino F, Ieni A, Monaco F, Caramori G, Sohal SS, Bracke KR, Wark PA, Adcock IM, Miyake K, Sin DD, Hansbro PM. TLR7 promotes smoke-induced experimental lung damage through the activity of mast cell tryptase. Nat Commun. 14(1):7349. doi:10.1038/s41467-023-42913-z. 2023

Chowdhury SI, Inui M, Yamazaki T, Tomono S, Takagi H, Biswas M, Saitoh SI, Miyake K, Akashi-Takamura S. The anti-TLR4 monoclonal antibody Sa15-21 enhances inflammatory cytokine production in LPS-stimulated macrophages. FEBS Lett. 597(9): 1246-1260. doi: 10.1002/1873-3468.14619. 2023

Kuchitsu Y, Mukai K, Uematsu R, Takaada Y, Shinojima A, Shindo R, Shoji T, Hamano S, Ogawa E, Sato R, Miyake K, Kato A, Kawaguchi Y, Nishitani-Isa M, Izawa K, Nishikomori R, Yasumi T, Suzuki T, Dohmae N, Uemura T, Barber GN, Arai H, Waguri S, Taguchi T. STING signalling is terminated through ESCRT-dependent microautophagy of vesicles originating from recycling endosomes. Nat Cell Biol. 25(3):453-466. doi: 10.1038/s41556-023-01098-9. 2023