3-C-W46-7-O/P  Arid5A is an IL-6 mRNA stability protein. Chlorpromazine mediates its inhibitory effect on IL-6 production in macrophages through inhibition of Arid5A expression. Masuda Kazuya¹, Ripley Barry¹, Nishimura Riko², Takeuchi Osamu³, Mino Takashi³, Standley Darin⁴, Kishimoto Tadamitsu⁵ (¹Immune Regulation, Osaka University, ²Departments of a Molecular and Cellular Biochemistry, Osaka University, ³Institute for Virus Research, Kyoto University, ⁴System Immunology, Osaka University)

In our previous study we demonstrated that chlorpromazine (CPZ) specifically inhibits LPS-induced IL-6 but not TNF, IL-12 and IL-10 production in macrophages. Here we studied the molecular mechanism underlying this inhibitory effect. We found that CPZ destabilizes LPS-induced IL-6 but not TNF-α mRNA in macrophages, independently of the NF-κB pathway. Mass spectral analysis showed that CPZ suppresses the binding activity of Arid5A to the IL-6 3’ UTR. Consequently, knockdown of Arid5A in LPS-treated macrophages inhibited the production of secreted IL-6 but not TNF-α. Moreover, overexpression of Arid5A enhanced IL-6 mRNA stability in LPS-treated macrophages. Arid5A thus functions to stabilize IL-6 mRNA, through its binding to the 3’ UTR of the RNA molecule. Taken together, these results demonstrate that the specificity of CPZ-induced inhibition of IL-6 production in macrophages is mediated via inhibition of LPS-induced Arid5A expression, and in turn, reduced IL-6 mRNA stability. Interestingly, we also found that Arid5A expression is induced under Th17 polarizing conditions (IL-6+Thp-1 stimulation) in naive T cells. Arid5A thus not only plays an important role in IL-6 mRNA stability but also has a key role in the function and differentiation of Th17 cells. In the future, Arid5A may be a therapeutic target of autoimmune diseases, such as rheumatoid arthritis.

3-C-W46-9-P  Auto-antibody production by murine B-1a cells stimulated with Helicobacter pylori urease through TLR2 signaling. TAKAHASHI Hidemi¹, KOBAYASHI Fumiko², KOIKE Eri³, YAMANISHI Shingo¹, NOROSE Yoshikiko¹, NAKAGAWA Yokho¹ (¹Department of Microbiology and Immunology, Niigata Medical School, ²Department of Pediatrics, Niigata Medical School)

Helicobacter pylori (H. pylori) infection is associated with several autoimmune diseases, in which autoantibody-producing B cells must be activated. Among the B cells, CD5-positive B-1a cells from BALB/c mice were confirmed to secrete autoantibodies when co-cultured with purified H. pylori urease in the absence of T cells. To determine the mechanisms for autoantibody production, CD5-positive B-1a cells were sorted from murine spleen cells and stimulated with either purified H. pylori urease or plate-coated H. pylori and autoantibody production was measured by enzyme-linked immunosorbent assay (ELISA). Complete urease was not secreted from H. pylori but was visually expressed over the bacteria like endotoxin. Urease-positive plate-coated H. pylori stimulated B-1a cells to produce autoantibodies, although urease-deficient isotype-matched H. pylori did not. Autoantibody secretion by B-1a cells was inhibited when bacteria were pretreated with anti-H. pylori urease-specific antibody having neutralizing ability against urease enzymatic activity but not with anti-H. pylori urease-specific antibody without neutralizing capacity. The B-1a cells externally express various toll-like receptors (TLRs); TLR1, TLR2, TLR4, and TLR6. Among these TLRs, blocking of TLR2 on B-1a cells with a specific monoclonal antibody (mAb), T2.5, inhibited autoantibody secretion when B-1a cells were stimulated with plate-coated H. pylori or H. pylori urease. Moreover, B-1a cells from TLR2 knockout mice did not produce these autoantibodies. The present study provides evidence that functional urease expressed on the surface of H. pylori will directly stimulate B-1a cells via innate TLR2 to produce various autoantibodies and may induce autoimmune disorders.

3-C-W46-10-P  Effect of Cigarette Smoking on Infiltration of Neutrophils to Pulmonary by LPS SAKASI Kazuma¹, KAWAZOE Ayaka¹, HIRONO Yuriko², NOSE Masahito¹, SHIGEYOSHI Eri¹, TANAHASHI Tatsuya², SAKURA Makiko¹, TAKEUCHI Mioru² (¹Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, ²Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, ³Laboratory of Infection and Prevention, Institute for Virus Research, Kyoto University)

The immune cell activation is tightly controlled at the transcriptional and post-transcriptional levels. Recently studies revealed the importance of post-transcriptional regulation in cytokine production in innate immune cells in response to Toll-like receptor (TLR) stimulation, and in the prevention of unnecessary inflammation. We have previously identified an RNAase named Regnase-1 destabilizing mRNAs such as IL-6 and IL-12. Regnase-1 is critical for suppressing the development of an inflammatory disease in mice. Nod2 binding protein 4 (Nbp1) harbors a N-terminal K homology domain and a C-terminal nuclease domain with a remote homology with that of Regnase-1. Nbp1 has been shown to be localized to the nucleus, in contrast to the Regnase-1 localization in cell cytoplasm. In this study, we show that Nbp1 is critical for suppressing production of cytokines in macrophages. TLR-mediated expression of cytokine mRNAs such as IL-6 was enhanced in Nbp1 KO macrophages, though activation of NFκB was unaffected. Re-expression of wild-type, but not nuclease-dead mutant (D621N), Nbp1 in Nbp1-/- macrophages suppressed IL6 production. Interestingly, Nbp1 KO mice showed glomerulonephritis, infiltration of lymphocytes in several tissues, and produced autoantibodies. These data indicate that Nbp1-mediated nuclear RNA decay is important for the regulation of innate immune system and prevention of the development of autoimmune inflammation in vivo.

3-C-W46-11-P  Effect of Cigarette Smoking on Infiltration of Neutrophils to Pulmonary by LPS SAKASI Kazuma¹, KAWAZOE Ayaka¹, HIRONO Yuriko², NOSE Masahito¹, SHIGEYOSHI Eri¹, TANAHASHI Tatsuya², SAKURA Makiko¹, TAKEUCHI Mioru² (¹Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, ²Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, ³Laboratory of Infection and Prevention, Institute for Virus Research, Kyoto University)

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