

## Journal Club

PREPRINT WATCH




## REPEAT ELEMENTS AMPLIFY TLR SIGNALING

Recognition of pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) can activate a protective type I interferon response. There is accumulating evidence that transposable elements from the host genome, including endogenous retroviruses, *Alu* elements and long interspersed nuclear elements (LINEs), can also act as PRR ligands upon expression. So far, this has been observed during breach of cellular homeostasis, such as in viral infection, cancer, senescence and in autoimmune diseases. However, the expression of transposable elements has been considered a cause, rather than a consequence, of innate immune activation.

In a recent preprint (not peer reviewed), Rookhuizen et al. make the surprising observation that stimulation of Toll-like receptor 4 (TLR4) with bacterial lipopolysaccharides (LPS) triggers the expression of transposable elements, and in particular the retrotransposon LINE1. This involves the downregulation of the histone methyltransferase SUV39H1, which marks DNA regions for transcriptional repression. Importantly, by treating mouse bone marrow-derived dendritic cells with a purified TLR4 ligand, the authors avoided cell stress associated with infection or the use of transformed cell lines, which can impact transposable element expression and confound results.

What are the functional consequences of TLR4-driven expression of transposable elements? The authors show that de-repressed LINE1 retrotransposons are reverse-transcribed and the resulting cDNA triggers the cGAS–STING pathway, amplifying the interferon response induced by the original PAMP. In an in vitro model of influenza A infection, they find that blocking LINE1 transcription, reverse transcription, or cGAS–STING signalling enhances viral replication and reduces cell survival.

Going forward, it will be critical to understand to what extent these findings, primarily obtained with mouse cells, may translate to humans, especially as transposable elements are markedly different between species. Further, quantifying the relative contribution of this pathway to the total interferon levels measured in vivo upon TLR4 signalling will determine whether these results may have translational relevance. Reverse transcriptase inhibitors (RTi) targeting the activity of LINE1 reverse transcriptase have shown promising results for treating interferonopathies in Aicardi–Goutières syndrome, and the work of Rookhuizen et al. provides a rationale for exploring a broader application of RTi for modulating interferon signalling. Key outstanding questions concern the mechanistic pathways involved in SUV39H1 downregulation in response to TLR4 activation and in the subsequent rapid upregulation of transposable elements.

Timothy O'Donnell  and Nicolas Vabret  
OxMS Preprint Journal Club, Icahn School of  
Medicine at Mount Sinai, New York, NY, USA  
✉e-mail: [highlights@preprintclub.com](mailto:highlights@preprintclub.com)

**ORIGINAL ARTICLE** Rookhuizen, D. C. et al. Induction of transposable element expression is central to innate sensing. Preprint at bioRxiv <https://doi.org/10.1101/2021.09.10.457789> (2021)

**RELATED ARTICLE** O'Donnell, T. J. Oxford–Mount Sinai (OxMS) Preprint Journal Club. OxMS <https://www.preprintclub.com/2021-sept-rookhuizen> (2021)

## INNATE IMMUNITY

## Red blood cells join the ranks as immune sentinels

Red blood cells (RBCs) are typically considered to be immunologically inert. However, Lam et al. now show that mammalian RBCs bind cell-free DNA, which leads to phagocytosis of RBCs and innate immune activation in pathological settings.

A previous study from the same group detected intracellular expression of the nucleic-acid receptor Toll-like receptor 9 (TLR9) by RBCs and showed a role for RBCs in sequestering potentially harmful cell-free DNA during homeostasis. In this study, Lam et al. used antibodies to the TLR9 ectodomain to show that TLR9 can also be detected on intact, nonpermeabilized RBCs from humans, mice and chimpanzees. Furthermore, RBCs from healthy human donors were shown to bind bacterial genomic DNA from *Legionella pneumophila*, mitochondrial DNA (mtDNA) from

the malaria parasite *Plasmodium falciparum* and synthetic CpG DNA. As CpG-containing mtDNA is increased in the circulation during sepsis, the authors examined RBCs from critically ill patients with sepsis and showed that both surface TLR9 expression and mtDNA binding are increased compared with RBCs from healthy donors. Levels of mtDNA on RBCs were similarly increased in mouse models of sepsis, bacterial pneumonia and parasite infection.

So what is the effect of binding of excess CpG DNA to RBCs? High-dose CpG treatment in vitro led to marked alterations of RBC morphology and changes to the distribution of cytoskeletal and membrane proteins in a TLR9-dependent manner. CpG treatment of RBCs also led to loss of surface expression of the antiphagocytic signal CD47 in a dose-dependent and TLR9-dependent manner.

## NEUROIMMUNOLOGY

## Glia mend the gut

Vassilis Pachnis and colleagues have identified a crucial role for enteric glial cells (EGCs) in regulating intestinal homeostasis and immunity. They show that EGCs promote tissue integrity by responding to IFN $\gamma$  — when IFN $\gamma$  signalling is impaired in EGCs, mice develop exaggerated intestinal inflammation.

Using EGC-reporter mice, Progotzky et al. found that during infection with *Heligmosomoides polygyrus*, the settlement of helminth larvae in the tunica muscularis (TM) of the duodenum is associated with the proliferation and activation of EGCs in the vicinity of the worms. Bulk and single-cell RNA-sequencing analyses of EGCs from infected and uninfected mice indicated that during *H. polygyrus* infection, EGCs adopt an activated cell state that is characterized by the upregulation of cell-cycle genes, gliosis markers and IFN $\gamma$ -target genes, such as *Gbp10*, *Stat1* and *Cxcl10*.

Of note, a similar IFN $\gamma$  gene signature was found in EGCs isolated from patients with ulcerative colitis. Thus, the upregulation of an IFN $\gamma$  gene signature by EGCs is seen in both infectious and inflammatory pathologies in different segments of the mammalian gut.

The authors showed that EGCs express both *Ifngr1* and *Ifngr2* (which encode the IFN $\gamma$  receptor) and respond to IFN $\gamma$  in vitro by activating STAT1, upregulating *Cxcl10* and *Gbp10*, and proliferating. Strikingly, when *Ifngr2*<sup>ΔEGC</sup> mice — which



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Credit: S. Bradbrook/Springer Nature Limited

Consistent with the loss of CD47, mice infused with CpG-treated RBCs had higher levels of erythrophagocytosis by splenic red pulp macrophages than mice infused with control RBCs. Mice infused with CpG-treated RBCs also had neutrophil infiltration of the spleen, increased splenic expression of interferon signalling pathway genes and increased plasma levels of IFN $\gamma$  and IL-6, which are indicative of both local and systemic immune activation. In critically ill patients with sepsis, those who were anaemic had higher levels of RBC-associated mtDNA than those who were not anaemic, which also supports increased erythrophagocytosis in response to DNA binding by RBCs. In erythrocyte-specific *Tlr9*-knockout mice compared with wild-type mice, IL-6 levels were attenuated after CpG administration or in a caecal slurry model of sepsis.

have a conditional deletion of *Ifngr2* in EGCs — were infected with *H. polygyrus*, by 7 days post-infection (d.p.i.) they showed bleeding at sites of the worm and by 28 d.p.i. they exhibited markedly increased intestinal pathology, characterized by more and larger granulomas, higher accumulation of inflammatory cells and aberrant gut peristalsis. Helminth-infected *Ifngr2*<sup>ΔEGC</sup> mice also had fewer CD8<sup>+</sup> T cells at 7 d.p.i. than controls, but showed no differences in CD4<sup>+</sup> T cell,  $\gamma\delta$  T cell and natural killer cell numbers or in terms of worm or egg burdens.

To better understand the IFN $\gamma$ -EGC axis, the authors used UMAP analysis of TM cells from uninfected or helminth-infected control or *Ifngr2*<sup>ΔEGC</sup> mice. Interestingly, in uninfected *Ifngr2*<sup>ΔEGC</sup> mice, several cell clusters (including mesothelial cells, macrophages and fibroblasts) showed upregulation of inflammatory pathways. In line with this, ~25% of naive *Ifngr2*<sup>ΔEGC</sup> mice showed signs of TM inflammation. During helminth infection, both control and *Ifngr2*<sup>ΔEGC</sup> mice showed increased immune cell representation in the TM and induction of type 2 immune

Together, the results suggest that when plasma CpG DNA levels increase beyond homeostatic norms, such as during sepsis or infection, TLR9-dependent binding to RBCs results in erythrophagocytosis, with consequent anaemia and innate immune activation. Anaemia and high cytokine levels are common features of multiple inflammatory pathologies, so further investigation of this pathway could have important therapeutic implications. Indeed, this study also reported that in hospitalized patients with COVID-19 pneumonia, the amount of RBC-bound mtDNA correlated with both anaemia and disease severity.

Kirsty Minton

**ORIGINAL ARTICLE** Lam, L. K. M. et al. DNA binding to TLR9 expressed by red blood cells promotes innate immune activation and anemia. *Sci. Transl. Med.* **13**, eabj1008 (2021)

genes, such as *Arg1*, *Retnl* and *Chil3*. However, the induction of type 2 genes was markedly reduced in *Ifngr2*<sup>ΔEGC</sup> mice compared with controls, suggesting that gli-specific ablation of IFN $\gamma$  signalling impairs tissue healing responses in the gut.

Early in the infection, the authors observed that *H. polygyrus* drives the recruitment of IFN $\gamma$ -producing cells that promote upregulation of *Cxcl10* by EGCs. Notably, *Cxcl10*<sup>ΔEGC</sup> mice infected with *H. polygyrus* developed a similar exaggerated inflammatory pathology to that seen in the *Ifngr2*<sup>ΔEGC</sup> mice. Therefore, CXCL10 production by EGCs is an important component of the IFN $\gamma$ -driven tissue healing response during helminth infection. The authors suggest that this IFN $\gamma$ -EGC-CXCL10 axis could also be relevant to the pathogenesis of other gastrointestinal disorders, including inflammatory bowel disease.

Yvonne Bordon

**ORIGINAL ARTICLE** Progradzky, F. et al. Regulation of intestinal immunity and tissue repair by enteric glia. *Nature* <https://doi.org/10.1038/s41586-021-04006-z> (2021)

## IN BRIEF

### COVID-19

#### Foetal sex affects maternal and placental immune responses to SARS-CoV-2

Immune responses to SARS-CoV-2 show sex-specific differences, with males at higher risk of severe COVID-19. Now, Andrea G. Edlow and colleagues have examined whether foetal sex affects immune responses to SARS-CoV-2 in pregnant women. The authors examined maternal and placental immune responses in 38 women with mild or moderate COVID-19 during pregnancy, as well as a control cohort. They found reduced maternal SARS-CoV-2-specific antibody titres as well as reduced transplacental transfer of these antibodies in women carrying male foetuses. Moreover, they observed a sexually dimorphic expression of placental Fc receptors, differences in antibody fucosylation and an upregulation of interferon response genes in male placentas. These results demonstrate that foetal sex affects maternal humoral immune responses as well as placental innate and adaptive immune responses to SARS-CoV-2.

**ORIGINAL ARTICLE** Bordt, E. A. et al. Maternal SARS-CoV-2 infection elicits sexually dimorphic placental immune responses. *Sci. Transl. Med.* <https://doi.org/10.1126/scitranslmed.abi7428> (2021)

### COVID-19

#### SARS-CoV-2 Delta variant excels at membrane fusion, but not immune evasion

The SARS-CoV-2 Delta variant has become the dominant strain worldwide. It is around twice as transmissible as its ancestral strain, with a shorter incubation period and higher viral load during infection. Now, Bing Chen and colleagues show that mutations in spike protein of Delta allow for faster membrane fusion than Alpha, Beta, Gamma and Kappa variants, and that Delta is more efficient at infecting cells with very low expression of the ACE2 entry receptor. However, the mutations found in the Delta variant had less impact on its sensitivity to neutralizing antibodies compared to those of the Gamma and Kappa variants. Neutralizing antibodies predominantly target the N-terminal domain (NTD) or the receptor binding domain (RBD) of the spike protein. The authors found different arrangements of the antigenic surface of the NTD in the different variants, but only local changes in the RBD, indicating that therapeutic antibodies or universal vaccines should be targeted at the latter.

**ORIGINAL ARTICLE** Zhang, J. et al. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. *Science* <https://www.science.org/doi/10.1126/science.abl9463> (2021)

### COVID-19

#### Comparing neurological complications after COVID-19 vaccination and SARS-CoV-2 infection

A large, population-based study of over 30 million people in the UK examined rare adverse neurological events 28 days after vaccination with ChAdOx1nCoV-19, BNT162b2 or a positive test for SARS-CoV-2. Overall, the authors identified a small but increased risk of hospital admission for Guillain-Barré syndrome, Bell's palsy and myasthenic disorders after ChAdOx1nCoV-19 vaccination, and for haemorrhagic stroke after BNT162b2 vaccination. However, they found that infection carries a much greater risk of neurological complications. For example, the authors estimated 38 excess cases of Guillain-Barré syndrome per 10 million doses of ChAdOx1nCoV-19 and 145 excess cases per 10 million positive SARS-CoV-2 tests.

**ORIGINAL ARTICLE** Patone, M. et al. Neurological complications after first dose of COVID-19 vaccines and SARS-CoV-2 infection. *Nat. Med.* <https://doi.org/10.1038/s41591-021-01556-7> (2021)