



HAEMATOPOIESIS

Macrophage quality control of HSCs

Reporting in *Science*, Leonard Zon and colleagues describe how embryonic macrophages control the quality of haematopoietic stem cells (HSCs) in zebrafish, through interactions that lead to HSC proliferation or apoptosis.

High-resolution live imaging of zebrafish embryos showed that ~20–30% of HSCs in the embryonic niche are in contact with a macrophage at any one time and that 70% of HSCs experience macrophage contact over a 3-hour imaging period. Contacts lasted up to 45 minutes and led to either the uptake of small amounts of HSC material by the macrophage ('grooming') or HSC engulfment by the macrophage ('dooming'). Macrophage–HSC contact was shown to precede HSC division or death. Furthermore, when embryonic macrophages were depleted before 96 hours post-fertilization (by which point HSCs have doubled), there was a reduction in haematopoietic clonality in adult zebrafish, which suggests that macrophages regulate HSC clone number.

Proteomic analysis showed that calreticulin paralogues are expressed by HSCs that lead to their interaction with macrophages transcriptionally enriched for the calreticulin binding partners *lrp1ab* and *c1qa*. Calreticulin knock down reduced HSC–macrophage interactions, in particular 'dooming', and nearly all HSCs overexpressing calreticulin were 'doomed'. Depletion of embryonic macrophages or calreticulin knock down also reduced the fraction of proliferative HSCs in the embryonic niche and reduced the number of HSC clones in adult zebrafish. HSC proliferation was shown to be induced by macrophage-derived IL-1 β . Together, the results suggest that HSC–macrophage interactions promote the death or proliferation of HSCs — in a manner dependent on calreticulin expression level — and thus regulate HSC clonality into adulthood.

Finally, the authors showed a significant correlation between surface calreticulin intensity on HSCs and endoplasmic reticulum stress and the accumulation of reactive oxygen species (ROS). They conclude that stressed HSCs that have increased risk of DNA damage and dysfunction express high levels of calreticulin that promote their removal by embryonic macrophages. By contrast, moderate levels of calreticulin expression are required for HSCs to receive proliferative 'grooming' signals from macrophages.

Kirsty Minton

ORIGINAL ARTICLE Wattrus, S. J. et al. Quality assurance of haematopoietic stem cells by macrophages determines stem cell clonality. *Science* **377**, 1413–1419 (2022)

MUCOSAL IMMUNOLOGY

Feeding IgA⁺ plasma cells

IgA secreted by tissue-resident plasma cells at mucosal barriers such as the intestines helps to regulate populations of commensal bacteria to ensure a mutually beneficial relationship and maintain tissue homeostasis. A recent study in *Science Immunology* shows a major role for food intake in aligning the considerable metabolic requirements of IgA production with periods of increased activity.

Penny and Domingues et al. observed significant variation in faecal IgA levels of C57BL/6 mice at five time points over a 24-hour period, and showed that this occurred in the absence of time-of-day differences in the frequency or number of IgA⁺ plasma cells in the small intestine or colon. Instead, the intrinsic capacity of plasma cells to secrete IgA ex vivo varied according to the time of day at which they were purified. Bulk RNA sequencing of small intestinal IgA⁺ plasma cells purified at different times of day identified 2,713 genes

with oscillatory expression, enriched for pathways involved in protein translation and metabolic activity.

IgA⁺ plasma cells had significant oscillation of core clock genes such as *Arntl*, *Nr1d1* and *Per2*, showing that — similar to many other types of immune cell — they have a cell-intrinsic circadian rhythm. However, in mice in which *Arntl* was deleted in B cells and plasma cells, the diurnal oscillation of faecal IgA levels was retained despite the loss of some time-of-day differences in clock-associated and plasma cell-associated genes. The authors conclude that additional factors entrain the circadian function of plasma cells in the absence of the intrinsic clock.

IgA responses are highly sensitive to dietary changes, so the researchers looked at feeding-associated cues as one such potential additional factor. Mice fed during the dark phase of a 12-hour light:dark schedule had similar oscillations in faecal IgA to mice fed ad libitum (which have a

INNATE IMMUNITY

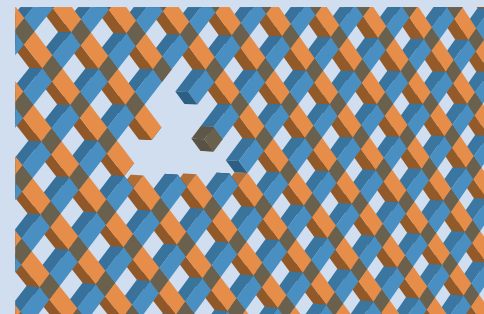
Disturbance of cytoskeleton primes RIG-I-like receptors

Michaela Gack and colleagues have found that disturbance of the cell cytoskeleton is a necessary priming step for activation of the RIG-I-like receptors (RLRs) RIG-I and MDA5. They show that remodelling of the actin cytoskeleton, which commonly occurs during virus infection, drives relocation of a PPP1R12C phosphatase complex to RIG-I and MDA5, where it dephosphorylates the RLRs to enable their activation.

RLRs are intracellular pattern-recognition receptors that sense RNA and upregulate antiviral responses via the MAVS signalling pathway. Dephosphorylation of caspase activation and recruitment domains (CARDs) in the amino-terminal of RLRs is mediated by the PP1 α and PP1 γ phosphatases and is necessary for RLR multimerization, MAVS binding and downstream signalling. However, PP1 α / γ have many other cellular

substrates, and it is not known how these phosphatases are specifically recruited to RLRs.

Previous work from the Gack laboratory had identified eight putative PP1 regulatory proteins; in the current study Acharya et al. used siRNA-mediated knockdown to determine if these proteins directly regulate RLRs. They found that silencing of PPP1R12C, but not the other candidate proteins, enhanced



Credit: Simon Bradbrook/Springer Nature Limited

IN BRIEF

IMMUNOTHERAPY

Boosting cytotoxic T cells for immunotherapy

Two papers in *Nature* provide insights into the synergistic activity of PD-1-targeted checkpoint inhibitors and IL-2 or IL-2 receptor (IL-2R) agonists. In a mouse model of LCMV infection, Hashimoto et al. show that the binding of IL-2 to CD25 (the IL-2R α chain) tweaks the differentiation programme of antigen-experienced PD-1⁺TCF1⁺ 'stem-like' CD8⁺ T cells. In contrast to PD-1 inhibition alone, which expands a population of transitory effector T cells that eventually become exhausted, PD-1 blockade combined with IL-2R signalling results in transcriptionally and epigenetically distinct T cells with superior antiviral activity. Codarri Deak et al. achieved similar effects with PD-1-IL2v, which combines PD-1 blockade with an agonist to IL-2R $\beta\gamma$. This molecule enables highly specific targeting of antigen-experienced PD-1⁺ T cells and avoids CD25-mediated side effects, such as the preferential activation of regulatory T cells and lung endothelial cells. PD-1-IL2v showed promising activity in preclinical cancer models, including a model of pancreatic cancer.

ORIGINAL ARTICLE Hashimoto, M. et al. PD-1 combination therapy with IL-2 modifies CD8⁺ T cell exhaustion program. *Nature* <https://doi.org/10.1038/s41586-022-05257-0> (2022) | Codarri Deak, L. et al. PD-1-cis IL-2R agonism yields better effectors from stem-like CD8⁺ T cells. *Nature* <https://doi.org/10.1038/s41586-022-05192-0> (2022)

IMMUNE MEMORY

How do smallpox-specific memory B cells survive?

Memory B cells (MBCs) can persist for a lifetime, but how they do this is poorly understood. Chappert et al. isolated vaccinia-specific MBCs from individuals who were vaccinated more than 40 years ago — and as smallpox was eradicated in 1980, these cells have not been re-stimulated, providing a unique opportunity to study their longevity. The antigen-specific cells were enriched in a splenic CD21^{hi}CD20^{hi} IgG⁺ MBC subset and had limited intra-clonal diversity. They had also undergone extensive affinity-based selection and had elongated telomeres. The analysis further suggested that early MBCs in germinal centres are imprinted with long-lasting potential through telomere elongation and that a regular transit into a splenic niche provides the crucial stimuli that enable these cells to stay functional over many decades.

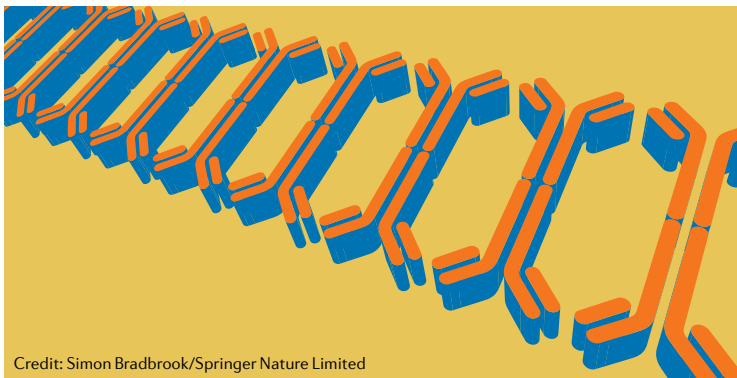
ORIGINAL ARTICLE Chappert, P. et al. Human anti-smallpox long-lived memory B cells are defined by dynamic interactions in the splenic niche and long-lasting germinal center imprinting. *Immunity* <https://doi.org/10.1016/j.immuni.2022.08.019> (2022)

NEUROIMMUNOLOGY

Effect of prenatal stress on the developing brain

Maternal environmental factors such as poor nutrition, immune activation and aberrant microbiome can affect the prenatal brain. Hayes et al. now investigate how the environment affects the microglia that infiltrate the neuroepithelium in early embryonic development. In a mouse model of maternal immune activation (MIA), the authors show that inflammatory stress leads to the long-term blunting of microglia immune reactivity in the adult offspring, with microglia showing changes in chromatin structure, transcription factor occupancy and transcriptional regulation. They also detected dysfunctional connectivity of the ventral striatal circuit in MIA-exposed offspring, but this could be averted by prenatal replacement of microglia with a physiological infiltration of naive microglia. Thus, prenatal stress can affect neuronal network formation via microglia.

ORIGINAL ARTICLE Hayes, L. N. et al. Prenatal immune stress blunts microglia reactivity, impairing neurocircuitry. *Nature* <https://doi.org/10.1038/s41586-022-05274-z> (2022)



Credit: Simon Bradbrook/Springer Nature Limited

largely nocturnal feeding pattern), whereas light-fed mice had a reversal of the IgA secretion pattern and of metabolism-associated gene expression by plasma cells. Moreover, mice fed a high-fat diet had a complete loss of IgA rhythmicity after 6 weeks, which suggests that nutrient availability might be a rate-limiting factor for antibody production. In support of this, the IgA secretory capacity of plasma cells *ex vivo* was sensitive to the availability of glucose and leucine in the culture medium.

Lastly, the authors identified IgA-dependent oscillations in commensal bacteria and their metabolism. Using mice that lack the ability to secrete antibodies, they identified several bacterial genera that lost circadian rhythmicity in the absence of IgA.

Mice lacking secreted antibody also had a near-complete loss of oscillatory bacterial expression of genes related to glycolysis and gluconeogenesis. Furthermore, time-of-day differences in faecal and blood glucose levels were attenuated in these mice.

Thus, the results suggest that increased nutrient availability after feeding enables intestinal plasma cells to secrete increased levels of IgA, which regulates commensal bacteria for optimal release of nutrients from the diet, as well as potentially other homeostatic functions.

Kirsty Minton

ORIGINAL ARTICLE Penny, H. A. et al. Rhythmicity of intestinal IgA responses confers oscillatory commensal microbiota mutualism. *Sci. Immunol.* **7**, eabk2541 (2022)

phosphorylation of RIG-I and MDA5 to a similar extent as PP1 α/γ deletion, suggesting PPP1R12C supports the dephosphorylation of RLRs. In agreement with this, depletion of PPP1R12C impaired IFN β induction in cells stimulated with synthetic dsRNA or infected with SARS-CoV-2, Zika virus or vesicular stomatitis virus (VSV). Similar findings of impaired IFN β induction and antiviral gene expression were seen in PPP1R12C knockout cells, and in response to VSV infection, Ppp1r12c-deficient mice showed impaired innate immune responses, enhanced viral replication and higher mortality.

Further experiments showed that infection with various RNA viruses causes PPP1R12C binding to RIG-I and MDA5. PPP1R12C also showed increased PP1 binding following virus infection, and the authors found that it recruits PP1 phosphatases to the RLRs through the formation of PP1-PPP1R12C-RLR complexes.

PPP1R12C regulates cytoskeleton dynamics as part of the myosin phosphatase complex; therefore, the authors hypothesized that actin cytoskeleton disturbance may displace

PPP1R12C from F-actin to promote PP1-PPP1R12C-RLR complex formation. They confirmed this idea using both viral and non-infectious triggers of cytoskeleton disturbance. Notably, the authors found that full activation of RLRs requires both RNA binding and actin cytoskeleton disturbance. They showed that inducible expression of immunostimulatory RNA in cells only led to antiviral gene expression if cells were also treated with agents that disturb the cytoskeleton and cause relocalization of PPP1R12C.

These findings challenge the current view that the presence of immunostimulatory RNA is sufficient for RLR activation. Instead, the authors propose that full RLR activation requires two key trigger steps: first, actin cytoskeleton disturbance to prime RLRs via PP1-PPP1R12C-RLR complex formation, and second the detection of immunostimulatory RNA.

Yvonne Bordon

ORIGINAL ARTICLE Acharya, D. et al. Actin cytoskeleton remodeling primes RIG-I-like receptor activation. *Cell* <https://doi.org/10.1016/j.cell.2022.08.011> (2022)